(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 October 2002 (17.10.2002)

PCT

(10) International Publication Number
WO 02/080953 A2

(51) International Patent Classification[7]: A61K 38/00

(21) International Application Number: PCT/NL02/00217

(22) International Filing Date: 3 April 2002 (03.04.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/NL01/00276 3 April 2001 (03.04.2001) NL

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EC, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 02/080953 A2

(54) Title: AT LEAST PARTIAL PREVENTION AND/OR REDUCTION OF CELLULAR DAMAGE IN TISSUE THAT HAS SUFFERED FROM OR IS SUFFERING FROM HYPOXIA AND/OR ISCHAEMIA AND/OR INFLAMMATION

(57) Abstract: The present invention provides means and methods for at least a partial prevention of cellular damage in disease resulting from hypoxia/ischaemia (H/I)-related inflammation, preferably due to occlusion of blood vessels. Administration of IFN type-I before, around or after the time of lifting of the ischaemia results in improved survival of cells downstream of the occluded blood vessel, presumable because the H/I-related inflammation is attenuated. Treatment results in less damage, better recovery and/or improved repair of the affected tissue.

Title: At least partial prevention and/or reduction of cellular damage in tissue that has suffered from or is suffering from hypoxia and/or ischaemia and/or inflammation.

The invention relates to the field of medicine. The invention more in particular relates to the treatment of diseases, more specifically diseases in which interruption of blood flow and/or activation/infiltration/proliferation of immune cells and/or inflammation has detrimental effects on tissue.

Interruption of blood flow is detrimental to tissue and in fact is the cause of a variety of human disease. Restoration of blood flow, spontaneous and/or treatment-induced can theoretically salvage all or part of the affected tissue. The success of this reperfusion in preventing further tissue damage is amongst others dependent on the severity of blood flow decrease and the time to restoration of blood flow to levels compatible with cell survival.

Treatment directed to increase blood flow - and/or spontaneous restoration of blood flow - to therapeutically relevant levels and treatment directed to protect cells from the results of the blood flow interruption is often counteracted by a phenomenon that we refer to as hypoxia/ischaemia (H/I) related inflammation. The H/I related inflammation adversely affects the survival of tissue after removal of the primary obstruction, or, when the obstruction has not (yet) been removed: the chance of survival of the tissue. This phenomenon is not only relevant in situations where blood supply is temporarily interfered with. A similar phenomenon is observed in situations where the demand for blood flow is (chronically) lower than the supply. Although the term hypoxia refers to situations of oxygen shortage, the phenomenon does not have to be related to the oxygen shortage. The term is here only used to indicate a situation in or after which the phenomenon is observed. The phenomenon may for instance also be induced by nutrient shortage. H/I related inflammation is a problem in a variety of human disease. The reasons for the detrimental effect of the phenomenon on the survival of

tissue after removal of the primary obstruction are not known. The effects may be the primary or secondary result of inflammation. It may also be the result of resistance to the increased blood flow following removal of the obstruction or be due to the quality of the remaining perfusion. Inflammatory like responses are typically observed in these situations. Various associations and explanations for the inflammatory reaction have been proposed, however as of yet this has not led to the development of improved therapies.

The present invention now provides a method for the treatment of H/I related inflammation in an individual comprising administering to said individual a therapeutically relevant dose of a type I interferon (IFN type-I) or a functional part, derivative and/or analogue thereof. Administration of said dose leads to reduced H/I related inflammation in said individual. The invention further provides a method for reducing cellular damage in tissue that has suffered from or is suffering from hypoxia and/or ischaemia and/or inflammation in an individual comprising administering to said individual a therapeutically relevant dose of IFN type-I or a functional part, derivative and/or analogue thereof. Survival of tissue displaying H/I related inflammation in the untreated individual, is improved in the comparable tissue in the treated individual. Less damage, better recovery or improved repair of the affected tissue is observed. One explanation is that as a result of the administration of a type I interferon, the inflammatory response is attenuated. H/I related inflammation does not have to be displayed by the individual at the time of administration. Prophylactic application is also beneficial. I.e. when it is expected that H/I related inflammation can occur in an individual said individual can be treated with a method of the invention and profit from the reduced damage, better recovery or improved repair of the affected tissue, as compared to the situation where no IFN type-I or functional part, derivative and/or analogue thereof, is given to said individual. As a non-limiting example this situation is illustrated for instance in surgery where one

or more parts of the body may suffer from reduced blood flow due to isolation from the circulation. With the phrase "treatment of H/I related inflammation" is meant that at least one effect of the phenomenon that we refer to as "H/I related inflammation" is at least in part reduced as a result of said treatment. Effects of the phenomenon are, for example, tissue damage, cell death or loss of function. Some or all of the results of the treatment may be due to the attenuation of the inflammatory response. Complete disappearance of, or protection against the inflammation as a result of treatment is possible.

H/I related inflammation can occur in many situations. A person that has not been able to breath oxygen for a limited amount of time, for instance, because the individual is in a situation where he/she has to be resuscitated, can be treated with a method of the invention and expect a beneficial effect on the overall outcome. Another likely embodiment of the invention is the treatment of traumatic brain injury (TBI), which can result from accidents like car crashes, falling, being hit on the head and the like. After traumatic brain injury blood flow is often reduced as a result of swelling of brain tissue or rupture of blood vessels or both. The present invention would improve the outcome by attenuating the H/I related inflammation following traumatic brain injury, and thus result in reduced damage, better recovery or improved repair of the tissue affected by the injury compared to the situation where no treatment is given.

In a preferred embodiment of the invention said H/I related inflammation is the result of a shortage in blood supply. A situation where H/I related inflammation can occur is very frequently a local problem. Therefore typically, said H/I related inflammation affects a part of the body of said individual, preferably due to an obstruction of a blood vessel. An obstruction can be a complete obstruction of a vessel allowing no passage of blood. An obstruction can also be an incomplete obstruction of a vessel that disables the flow of blood to meet the demand of blood flow. An obstruction can be in the form of a clot that clogs the vessel, or can be caused by the expansion of a lesion in the vessel

wall or by a spasm of the vessel. However, an obstruction can also be the closing of a vessel by force exerted from the outside for instance due to clasping or swelling of surrounding tissue. An obstruction can also be caused by a cut effectively interfering with proper blood flow. A non-limiting example of the previous is a spontaneous rupture of a major supplying brain vessel, such as occurring in sub-arachnoid haemorrhage.

In one aspect the invention therefore provides a method for the treatment of a H/I related inflammation in an individual, said individual comprising at least one blood vessel obstruction causing ischaemia (the supply of blood lags behind the demand of blood, whether due to obstructions, increased demand, lowered level of oxygen and/or nutrients in the blood or any other way) in tissue that is situated downstream from said obstruction, said method comprising administering to said individual a therapeutic dose of IFN type-I or a functional part, derivative and/or analogue thereof. A treatment of the invention preferably results in improved survival of cells in said individual, preferably, said cells comprises neuronal cells. In another aspect the invention provides a method for at least in part improving blood flow in post-ischaemic tissue, for example by attenuating the clogging of microvasculature by inflammatory cells adhering to the endothelium, comprising administering to an individual, having said tissue, IFN type-I or a functional part, derivative and/or analogue thereof. In yet another embodiment the invention provides a method for at least in part preventing cell death in post-ischaemic tissue comprising administering to an individual, having said tissue, IFN type-I or a functional part, derivative and/or analogue thereof. Preferably, said cell death is in part prevented in neuronal tissue.

In a preferred aspect of the invention said H/I related inflammation is restricted to the brain or spinal cord, the heart, a transplanted organ and/or a limb. A method of the invention can be used to improve survival of transplanted organs or parts thereof. In organ transplantation, reperfusion injury causes tissue damage immediately following transplantation, probably

due to the presence and/or invasion of inflammatory cells in the damaged area. This process has been implicated in the development of acute and chronic rejection of transplants. NF-kB is a transcription factor that upregulates adhesion molecules, eg ICAM-1, VCAM-1, and E-selectin, following reperfusion. Systemic treatment with sulfasalazine, a potent inhibitor of NF-kB, was shown to decrease adhesion molecule expression, decrease reperfusion injury, and prolong allograft survival in rat cardiac transplants. IFN type-I reduces the expression of adhesion molecules, (eg ICAM-1 or VCAM-1), and can be administered to an individual according to a method of the invention to prolong allograft survival. One explanation for at least part of the beneficial effect of IFN type-I in this embodiment is that IFN type-I inhibits the induced production of IL-8 via transcriptional inhibition of IL-8 gene expression (Oliveira et al., 1992; Oliveira et al., 1994). Ischaemia-reperfusion injury in rat small intestine can be attenuated by administration of antibodies against IL-8 (Tsuruma et al., 1998).

In a preferred embodiment, said H/I related inflammation is restricted to a part of the brain and/or the heart. Particularly the brain is preferred because the administration of IFN type-I or a functional part, derivative and/or analogue thereof, has a dramatic effect on H/I related inflammation in this part of the body. Administration results in significantly less neuronal damage, increased cell survival and improved repair of the damaged area. The volume of the damaged area in the brain as a result of the administration is significantly reduced compared to untreated. One explanation for the observed effects of treatment is that inflammation in the affected area is attenuated significantly compared to untreated individuals.

In a preferred embodiment IFN type-I or a functional part, derivative and/or analogue thereof, is administered to patients in which the blood flow is insufficient in an area of the brain, preferably as the result of a stroke or

trauma. In cases where an obstruction of a vessel is the reason for the insufficient blood flow it is preferred that the compound is administered as soon as possible, and preferably even before occurrence of said obstruction. Administration can prolong the time window during which the (partial) removal of the obstruction is beneficial. In this case administration can be continued until a more or less stable situation has been arrived at. In cases where hypoxic/ischaemic tissue is reperfused, or perfused to a larger extent, a stable situation typically is arrived at within one week after the increased supply of blood flow was initiated. Nevertheless, treatment for longer periods (up to several weeks), is beneficial by attenuating the more chonic phase of H/I related inflammation. More chronic effects could be mediated by downregulation of VCAM-1

It is thought that in the brain, ischaemia and trauma elicit an inflammatory response in which mediators (cytokines, chemokines and adhesion molecules) are released and immune cells infiltrate the injured brain. Upregulation of pro-inflammatory cytokines, chemokines and endothelial-leukocyte adhesion molecules starts soon after ischaemia and trauma and continues during the evolution of tissue injury. For leukocytes to gain access to their target tissues, a variety of signaling proteins and adhesion molecules act in concert to permit chemotaxis, endothelial cell attachment and transmigration. For example a rapid overproduction of TNF-alpha leads to the stimulation of adhesion molecule expression with subsequent accumulation of leukocytes in the ischaemic focus. Focal adhesion of leukocytes to the endothelium is a step in inflammation and certain vascular disease processes. P- and E-selectin and ICAM-1, expressed by activated endothelium, and the leukointegrin CD11/CD18 expressed by activated leukocytes, have been shown to contribute to this adhesion. E-selectin and ICAM-1 are synthesized by stimulation with cytokines such as IL-1beta and TNF-alpha. Immunoreactivity to ICAM-1 and CD11/CD18 has been demonstrated after 60 min transient focal ischaemia in the rat, in both core and penumbra,

increasing from 3 to 24h after reperfusion. Leukocytic infiltration in this example was seen in the ischaemic areas from 12 to 24h after reperfusion. As mentioned above, effects of IFN type-I observed in the present invention can be the result of a reduction of inflammation responses in the affected areas. A possible mechanism by which inflammation can be reduced is by reducing the role of leukocytes. According to this view, leukocyte involvement in the pathogenenesis of cerebral ischaemic damage is as follows: exacerbation of blood-brain barrier- (BBB) or parenchymal injury via hydrolytic enzyme release, oxygen radical production and lipid peroxidation, and/or a reduction in cerebral blood flow by vessel plugging and/or release of vasoconstrictive mediators such as for example endothelin.

The present invention is suited for at least in part reducing the negative effects oftraumatic brain injury. Administration of IFN type-I reduces the volume of the damaged area, decreases cell death and enhances the recovery and/or repair of damaged tissue compared to untreated individuals. One explanation for the beneficial effects in traumatic brain injury is that the blood-brain-barrier is fortified. Dysfunction of the blood-brain barrier is mediated by chemokine release (eg IL-8), upregulation of adhesion molecules (eg ICAM-1, VCAM-1) and intracerebral neutrophil accumulation. IFN type-I can directly downregulate IL-8 via transcriptional inhibition of IL-8 gene expression and downregulates adhesion molecules. In patients with severe TBI, it was found that elevated cerebrospinal fluid IL-8 and soluble (s)ICAM-1 correlated with BBB-dysfunction. IL-8 and sICAM-1 were upregulated for 19 days after severe TBI, whereas their stimulator TNF-alpha was upregulated for 9 days. The same holds true for critical limb ischaemia.

The mechanisms behind the beneficial effects of IFN type-I administration may be different for different diseases. I.e reduction in brain damage could at least in part be due to a different effect than beneficial effects in the treatment of listed diseases affecting other parts of the body.

IFN type-I has pleiotropic effects. Several possible ways in which IFN type-I might act are listed below.

IFN type-I stimulates IL-10 production: IL-10 is a classic anti-inflammatory cytokine. Administration of IL-10 alone is enough to reduce infarct size after permanent MCA-occlusion (Spera et al., 1998) and IL-10 plus mild hypothermia (but not hypothermia alone) provides long-lasting protection to CA1 hippocampus following transient global ischaemia (Dietrich et al., 1999). IFN type-I stimulates IL-10 production in an in vitro microglia-T-cell interaction model (Chabot and Yong, 2000). Liu et al., showed that monocytes in vitro and in vivo in MS patients produced more IL-10 following IFN type-I treatment (Liu et al., 2001). In a murine model of experimental autoimmune encephalomyelitis (EAE), an animal model sharing many features with MS, Tuohy et al. found that IFN type-I significantly skewed the response to the priming immunogen toward an increased production of IL-10 and a concurrent decreased production of IL-12 and that this was accompanied by an aborted development of epitope spreading (Tuohy et al., 2000). Others have shown that in clinical MS, levels of IL-10-secreting monocytes were augmented during IFN type-I treatment (Ozenci et al., 1999) and that IL-10 levels were elevated during resolution of lesions in IFN type-I treated patients (Waubant et al., 2001b). IL-10 can suppress the production of a variety of pro-inflammatory molecules, including TNF-alpha, IL-1beta, interferon-gamma and IL-6 (Fischer et al., 2001; Plunkett et al., 2001; Szczepanik et al., 2001). It diminishes neutrophil chemotaxis via IL-8 suppression (Howard and O'Garra, 1992; Ehrlich et al., 1998), it interferes with macrophage activity through inhibition of macrophage inflammatory protein-1 (MIP-1) expression and deactivates monocytes (Geng et al., 1994).

IFN type-I counteracts the pro-inflammatory cytokines TNF-alpha and IL-1beta: Numerous studies have shown the deleterious properties of TNF-alpha and IL-1beta in ischaemic stroke(Barone and Feuerstein, 1999). IFN type-I may attain its neuroprotective potential by combatting these key inflammatory cytokines: it has been shown in vitro (Chabot et al., 1997; Chabot and Yong, 2000; Jungo et al., 2001; Ossege et al., 2001), in mice (Bosca et al., 2000) and in blood from MS patients (Gayo et al., 1999; Perini et al., 2000; Ossege et al., 2001) that IFN type-I counteracts TNF-aplha- and IL-1beta actions by reducing their upregulation and by inducing the IL-1 receptor-antagonist, as well as inducing soluble TNF-alpha-receptor I and II (Perini et al., 2000).

IFN type-I attenuates expression of the chemokines IL-8, MCP-1, RANTES and MIP-1alpha: IFN type-I has been shown to inhibit the induced production of IL-8 via transcriptional inhibition of IL-8 gene expression (Oliveira et al., 1992; Oliveira et al., 1994). IL-8 (also termed CINC in rats) is a key mediator of leukocyte recruitment after ischaemia/reperfusion injury. The chemokine RANTES (regulated upon activation, normal T-cell expressed and secreted) triggers monocyte arrest on inflamed and atherosclerotic endothelium (von Hundelshausen et al., 2001), and Bona et al., showed in a neonatal hypoxia/ischaemia model that mRNA induction for MIP-1alpha and RANTES preceded the appearance of lymphocytes, microglia/macrophages, and natural killer cells in the infarct area (Bona et al., 1999). Zang et al., demonstrated that in vitro exposure of T cells to IFN type-I selectively inhibited mRNA expression for RANTES and MIP-1alpha and their receptor CCR5 and that T cell surface expression of CCR5 was significantly reduced in MS patients treated with IFN type-I, correlating with decreased T cell transmigration toward RANTES and MIP-1alpha (Zang et al., 2001). Iarlori et al, showed that in vivo RANTES production is reduced in sera from MS patients treated with IFN type-I (Iarlori et al., 2000). Monocyte

chemoattractant protein-1 (MCP-1) is downregulated by IFN type-I in experimental autoimmune neuritis (Zou et al., 1999) inhibiting migration of inflammatory cells into the nervous tissue. It was demonstrated that IFN type-I treatment abrogated RANTES-, MIP-1alpha- and MCP-1 induced migration of monocytes across a matrix of fibronectin, an extracellular matrix molecule and a major component of the endothelial basement membrane, through an effect on matrixmetalloproteinase-9 (MMP-9) (Stuve et al., 1997). IL-8 causes neutrophils to secrete MMP-9 (Masure et al., 1991). MIP-1alpha, MIP-1beta and RANTES enhance T-cell secretion of MMP-9. IFN type-I may not only reduce IL-8, MIP-1alpha and RANTES, it has also been shown to upregulate the naturally occurring inhibitor of MMPs, TIMP-1 (Waubant et al., 2001a). Together these mechanisms prevent trafficking and infiltration of pro-inflammatory cells into the lesions site. Moreover MMPs have been shown to be key players in thrombolysis-associated hemorrhagic transformation after focal ischaemia(Sumii and Lo, 2002). Thus the present invention, by reducing MMP-activity, is likely to reduce bleeding, either spontaneous or after thrombolysis, and can thus increase the safety and extend the time-window for thrombolytic therapy.

IFN type-I prevents the upregulation of several cell adhesion molecules: Not just via preventing the upregulation of pro-inflammatory cytokines but also by directly downregulating or inhibiting the increase in levels of ICAM-1 (on BMECs in vitro (Defazio et al., 2000; Trojano et al., 2000)), of MHC class-II molecules (which might also act as lymphocyte adhesion receptors) (Huynh et al., 1995; Miller et al., 1996) and of Beta1-integrin (or VLA-4) (Soilu-Hanninen et al., 1995; Muraro et al., 2000). Furthermore, it has been shown that increasing soluble VCAM-1 (sVCAM) release during active inflammation could be a mechanism by which IFN type-I treatment exerts protective effects in multiple sclerosis patients (Kallmann et al., 2000; Calabresi et al., 2001) since

sVCAM can bind and downregulate Beta1-integrin thus interfering with lymphocyte-adhesion to mVCAM (Calabresi et al., 1997).

IFN type-I decreases iNOS activity: Induction of iNOS is a well known aggravating factor mediating delayed ischaemic brain damage and its selective inhibition has been shown to be neuroprotective. Hua et al. found that in primary human astroglial cell cultures IFN type-I was a selective and potent inhibitor of IL-1beta- or interferon-gamma-induced iNOS expression (Hua et al., 1998). The same was found in a human astrocytoma cell line (Guthikonda et al., 1998) and murine macrophages (Deguchi et al., 1995; Lopez-Collazo et al., 1998).

IFN type-I upregulates HIF-1alpha and TGF-beta: Der et al., illustrated the pleiotropic nature of IFN type-I using oligonucleotide-arrays in a human HT1080 cell line, identifying over 300 genes that are upregulated at least twofold after IFN type-I-stimulation (Der et al., 1998). Amongst these: hypoxia inducible factor-1alpha (HIF-1alpha), which, under hypoxic conditions, activates the transcription of genes encoding erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor (VEGF), and other genes whose protein products increase $O_2$ delivery or facilitate metabolic adaptation to hypoxia. HIF-1alpha signaling might however also play a role in p53-mediated apoptotic cell death (Halterman et al., 1999) which seems to indicate that HIF-1alpha only induces transcription of protective genes in more penumbral regions where hypoxic stress is not too severe. IFN type-I-alpha induced the mRNA expression of TGF-beta and TGF-beta-R-II in vivo (Ossege et al., 2001) and acutely increased blood levels of TGF-beta.(Nicoletti et al., 1998). TGF-beta displays multiple anti-inflammatory activities; pretreatment reduced infarct size and increased cerebral blood flow in rabbit stroke model (Gross et al., 1993) and intraventricular injection 2

hours after hypoxic-ischaemic injury reduced cortical infarction in the rat (McNeill et al., 1994).

The reasonings given above are given only to provide the reader with a possible mechanism. The striking effects of IFN type-I or a functional part, derivative and/or analogue thereof on reducing damage and improving recovery and/or repair of tissue affected by H/I related inflammation, as demonstrated in the present invention, can also be caused through an entirely different mechanism.

The invention further provides a use of IFN type-I or a functional part, derivative and/or analogue thereof for the preparation of a medicament for the treatment of H/I related inflammation. Preferably, said inflammation is correlated with hypoxia and/or ischaemia. More preferably, wherein said impairment is in the brain.

In yet another aspect the invention provides a use of IFN type-I or a functional part, derivative and/or analogue thereof for the preparation of a medicament for the treatment of impairment of blood flow recovery. Preferably, said impairment is correlated with hypoxia and/or ischaemia. Preferably, said impairment is in a capillary vessel. Preferably, said impairment is in the brain.

The invention further provides a use of IFN type-I or a functional part, derivative and/or analogue thereof for the preparation of a medicament for at least in part preventing cell death in post-ischaemic tissue. Preferably, wherein said impairment is in the brain.

The present invention also provides means and methods for at least in part preventing ischaemia-related damage to tissue. Reduction in tissue

damage is possibly contributed to by, the result of reduced micro-vascular clogging, which occurs in situ in microvessels downstream from the site of occlusion. Reduced micro-vascular clogging prevents deterioration of blood flow in the microvasculature downstream of the ischaemia upon the (partial) removal of the primary occlusion. In one aspect the invention therefore provides a method for at least in part improving blood flow in tissue that has suffered or is suffering from ischaemia, comprising administering to an individual, having said tissue, IFN type-I or a functional part, derivative and/or analogue thereof. IFN type-I or a functional part, derivative and/or analogue thereof may be administered before the cause for the ischaemia is removed. Preferably, said IFN type-I or a functional part, derivative and/or analogue thereof is given as soon as possible, preferably together or immediately after treatment given to (partially) remove the primary occlusion is also possible.

The present invention is particularly suited for reducing damage and improving recovery and/or repair of the affected tissue in the brain. The volume of the damaged area is decreased compared to untreated cases. In addition the amount of in particular, neural cell death is decreased. IFN type-I administration is suited for the treatment of effects caused by any type of violence to the head, including but not limited to traumatic brain injury, frost damage, stroke and intracranial bleedings (eg: subarachnoid haemorrhage, thrombolytica-induced, etc.) IFN type-I or said functional part, derivative and/or analogue thereof is preferably administered as soon as possible after the cause of the damage has taken place. In this way medication or other methods to remove the obstruction is given a larger time window for effect. Thus improving the chances of recovery for a patient. Administration may also be in anticipation of the cause or as prophylactic. In some diseases caused by occlusion of blood vessels, the current perception is that there is no urgency in giving treatment since there is no effective way to prevent the tissue damage.

An example of such a disease is stroke. Very often, the fact that a patient had a stroke is not considered reason for immediate treatment the patient.

It is generally thought that damage inflicted by the stroke is already irreversible by the time a patient is hospitalized and available for treatment. With the present invention it is apparent that there is a good reason for rapid treatment of patients. With the means and methods of the invention it is possible to at least in part reduce the extent of tissue damage. The invention therefore further provides a method for at least in part preventing cell death in post-ischaemic tissue comprising administering to an individual, having said tissue, IFN type-I or a functional part, derivative and/or analogue thereof.

IFN type-I is currently used in the chronic treatment of multiple sclerosis (MS) patients and has been reported to cause systemic side-effects in this patient group. The systemic reaction is usually described as flu-like. Fever, chills, muscle aches, sometimes headaches, often commence a few hours after the drug is injected and last for some hours. For non-chronic cases, administration of IFN type-I can be discontinued after a relatively short period, often no longer than 1 week after the onset of the disease. In situations where prolonged administration is desired the side effects of the treatment can be tolerated.

Type I interferons encompass interferon alpha, interferon beta, interferon omega, and so-called consensus type interferon or hybrid types interferon (see for review Viscomi GC (1997) Structure-activity of type I interferons. Biotherapy 10:59-86.) In a preferred embodiment said IFN type-I comprises interferon β or a functional part, derivative and/or analogue thereof. A functional part of IFN type-I is a part of IFN type-I comprising the same post-ischaemic damage reduction activity in kind as IFN type-I itself. The amount of activity of such a part may differ from the activity of the complete

protein. A person skilled in the art is capable of generating a suitable derivative of IFN type-I. Derivatives can be for instance be obtained by conservative amino acid substitution. Indeed currently prescribed human interferons differ slightly in amino acid sequence from natural human interferons. A suitable part of IFN type-I is for instance a part with an altered glycosylation pattern or a part that is non-glycosylated. Glycosylation can be prevented by removing or altering a glycosylation site of the molecule. If the generation of such a (partially) deglycosylated IFN type-I requires alteration of the amino acid composition than such a deglycosylated IFN type-I is derivative of a functional part of IFN type-I. A functional part, derivative and/or analogue of IFN type-I comprises the same activity in kind not necessarily in amount.

In general, IFN type-I may modulate the profile of cytokine production toward that of the anti-inflammatory phenotype (for instance by upregulation of IL-10), and this appears to occur in the systemic circulation and within the CNS.

For suitable doses ranges and formulations of IFN type-I or a functional part, derivative and/or analogue thereof one may turn to literature regarding the use of said compound in humans in other disease areas such as multiple sclerosis. Additionally, dose finding studies can give suitable therapeutic doses for the present invention. By way of example a dose of 8M IU Betaseron, s.c., every other day; a currently accepted dosage regime for relapsing remitting MS. A 1.6 M IU dosage every other day was well tolerated by the patient(Arnason, 1999). Furthermore, up to 176 million IU (5.5 mg of protein) has been injected .i.v. in patients, three times a week, without severe side-effects. This is > 3 times higher than the dose per kg bodyweight demonstrated to be effective in the present invention after s.c. injection. Moreover, since also the bio-availability of about 50% of s.c. injected IFN type-I is much lower than that of directly i.v. injected IFN type-I, there is broad

treatment window within which IFN type-I administration can be varied to reach optimal individual effect.

The administration of IFN type-I or a functional part, derivative and/or analogue thereof, can be combined with on or more other compounds. Preferably said compound blocks the upregulation of adhesion molecules on endothelium or on the cells they would bind. However antibody against these receptors, shielding it and preventing binding or a cytokine-antagonist that inhibits the activity of cytokines regulating adhesion molecule expression or cytokines that mediate chemotaxis is also suitable. IFN type-I may also be combined with thrombolytic therapy. Attenuation of H/I related inflammation can add to the effectiveness of thrombolytic therapy and extend the time window for such treatment: For example, Zhang et al., found that co-treatment of rtPA with either anti-ICAM-1 antibody or with antileukocyte adhesion antibody (anti-CD18) resulted in a significant reduction in lesion volume compared with rats treated with rtPA only (Zhang et al., 1999a; Zhang et al., 1999b). The findings of Bowes et al., with antibodies against the same adhesion molecules in a rabbit cerebral embolism stroke model suggested that adjuvant anti-leukocyte adhesion-therapy increases the postischaemic duration at which thrombolytic therapy remaines effective (Bowes et al., 1995). Therefore, in a preferred embodiment IFN type-I or a functional part, derivative and/or analogue thereof is combined with treatment given to remove an obstruction from a vessel. Preferably, said treatment comprises administration of a thrombolyticum, such as for example tPA or similar compounds, or ultra-sound-mediated clot-removal. In another preferred embodiment IFN type-I is given together with neuroprotective drugs, such as erythropoietin or dihydroascorbic acid. Combinations may exert a synergistic effect on tissue damage reduction, cell survival and repair of tissue. In yet another preferred embodiment IFN type-I is given together with drugs that can ameliorate the side-effects of IFN type-I-treatment.

All type-I interferons (interferon-alpha, interferon-beta, interferon-omega, hybrids and consensus version thereof) share a common receptor through which at least some of their effect are mediated (i.e. composed of the α/β interferon receptor (IFNAR)-1 and IFNAR-2 chains (Uze et al, 1990, Cell 60,225-234; Novick et al, 1994, Cell 77, 391-400; Domanski et al 1995, J. Biol. Chem. 270, 21606-21611) The type-I-interferon-receptor can be targeted to develop compounds with similar activity as type I interferons. For the present invention such compounds are considered analogues of IFN type-I. . In the present invention interferon-beta was used to demonstrate the effectiveness of activating the IFN type-I-R1, but activating the receptor using any other compound (for example, interferon-alpha) is also effective.

Effectiveness of IFN type-I has been demonstrated using recombinant rat-IFN type-I in rats. The same recombinant rat-IFN type-I is also effective against experimental MS in rats, murine-IFN type-I is effective against MS in mice, both human- and primate-IFN type-I is effective against experimental MS in primates and recombinant human-IFN type-I has shown efficacy against MS in humans. Also, the occasional occurrence of a necrotic patch at the site of IFN type-I injection in rats, is a side-effect also seen in humans. These lines of evidence indicate a conserved mode of action of IFN type-I in these different species.

In one aspect the invention provides a use of IFN type-I or a functional part, derivative and/or analogue thereof for the preparation of a medicament for the treatment of H/I related inflammation. The invention also provides a use of IFN type-I or a functional part, derivative and/or analogue thereof for the preparation of a medicament, said medicament capable of at least in part preventing cell death in post-ischemic tissue. Preferably, said

impairment is in microvessels. More preferably, wherein said impairment is in the brain.

Examples

Example 1

Materials and Methods

Animal model

Male Fischer rats (F344/Ico, Iffa-Credo Broekman, Someren, The Netherlands) aged 8-12 weeks were used in all experiments. Animals had free access to standard laboratory chow and water. Anesthesia was induced by i.p. injection of a mixture of 0.5 ml/kg fentanyl citrate (0.315 mg/ml) and fluanisone (10.0 mg/ml), and 0.5 ml/kg midazolam (5.0 mg/ml), followed by s.c. injection of 0.1 ml/kg (0.5 mg/ml) atropine sulfate and i.p. injection of 0.5 ml/kg gentamicinsulfate (10 mg/ml). The animals were endotracheally intubated and mechanically ventilated with $O_2/N_2O$ (30/70 v/v). Body temperature was maintained at 37.0 ± 0.5 °C by means of a feedback-controlled heating pad. During surgery and NMR-measurements anesthesia was continued by adding 1-1.5% halothane to the $O_2/N_2O$ mixture. After surgery animals received 1 ml/kg buprenorfine s.c. (0.3 mg/ml).

Transient focal ischaemia was induced by unilateral tandem occlusion of the right common carotid artery (CCA) and middle cerebral artery (MCA) via a modification of the procedure as described by Brint et al. (1988). Briefly, a 2 mm ∅ hole was drilled just rostral to the oval foramen, exposing the right middle cerebral artery (MCA). After opening the dura and arachnoidea the MCA was transiently occluded using a vascular microclip (Codman).

Reperfusion was reinstated after 60 minutes under visual inspection by removing the clip.

Animals were treated with s.c. injections of 500.000 IU rrIFN-β in 1 ml phosphate buffered saline (PBS) once daily until 7 days after reperfusion (n=9). Control animals (n=5) received s.c injections with saline, starting 2 days prior to surgery up to 7 days after reperfusion. Treated animals were divided into three groups (n=3x3): treatment for group 1 started 2 days prior to surgery, treatment for group 2 started upon reperfusion and treatment for group 3 started 4 hours after stroke onset.

NMR experiments

NMR-experiments were performed on a 4.7T Varian horizontal bore spectrometer equipped with a gradient insert able to achieve gradients up to 220 mT/m in 300 μs. Rf-excitation and signal detection were accomplished by means of a Helmholtz volume coil (9 cm ∅) and an inductively coupled surface coil (2 cm ∅), respectively. Animals were positioned in an animal cradle and immobilized with ear bars. During the experiments exhaled $CO_2$ and rectal temperature were continuously monitored.

A single-scan diffusion-trace MRI-sequence (4 b's:100-1780 s/mm$^2$, repetition time (TR)=2s, echo time (TE)=100 ms, number of transients (NT)=2) was used to generate quantified images of tissue water trace apparent diffusion coefficient (ADC). $T_2$-weighted images were acquired using a multi-echo sequence (8 TEs: 17.5 ms + 7 x 17.5 ms, TR=2s, NT=2).

Diffusion-weighted and $T_2$-weighted-data sets (collecting 8 1.7 mm thick slices, 3.2x3.2 cm$^2$ FOV, 128x64 matrix, zero-filled to 256x256) were acquired at 1, 7 and 21 days after reperfusion.

Data analysis

ADC and $T_2$ maps were generated by mono-exponential fitting using IDL (Research Systems, Boulder, USA). Parametric images were analysed in

anatomic regions of interest using in-house software. Calculations of volumes of affected tissue were based on ipsilateral ADC or $T_2$ differing more than 20% (corresponding to >2x SD) from the mean value in the contralateral hemisphere. Statistical analysis was carried out using SPSS 9.0. Reported p-values correspond to two-tailed Student's t-tests or one-way ANOVA where appropriate.

Results

General Condition

After surgery, body weight of all animals showed an initial decrease in the first postoperative days, whereafter it increased.

In two treated animals a necrotic patch, about 7.5 mm in diameter, developed in the skin around the site of ifn-injection. The lesion was not painful to the touch and did not hinder the animals in feeding or other behavior. For the remaining days injection was continued at a different site, where, in these two animals, necrosis did not occur.

Magnetic Resonance Imaging

ADC images acquired at 1 day after reperfusion showed cytotoxic cell swelling, as evidenced by decreased ADC-values. In control animals both the cortex and a large part of the caudateputamen of the ipsilateral hemisphere were affected, while in interferon-treated animals a smaller part of the caudateputamen and no part or a only small rim of cortext showed cytotoxic cell swelling (see fig. 1). At this timepoint the volume of the lesion on ADC maps (ALV) was 76% smaller ($p<0.0005$) in treated animals compared to controls (see figs. 3 and 4). Also, in interferon-treated animals the severity of tissue damage, as determined from the severity of ADC-reduction, was 15% less ($p<0.002$) than in control animals (see fig. 5). There was no significant difference between the three treatment groups in terms of the $T_2$ data.

$T_2$ maps acquired at day 1 showed the development of vasogenic edema in the affected tissue, the volume of which was 64% smaller ($p<0.021$) in treated animals compared to controls (see figs. 2, 7 and 8). This difference is also demonstrated by the midline-shift caused by swelling of the brain tissue, which is absent or much less pronounced in treated animals compared to controls (see fig. 2). There was no significant difference between the three treatment groups.

ADC data acquired during the endpoint measurement on day 21 showed ADC values had increased to supranormal values, indicative of vasogenic edema and tissue loss (see figs 1, 3 and 4). At this timepoint the infarct as present on ADC maps was reduced by 82% ($p<0.0005$) in treated animals compared to controls.

$T_2$ map data acquired on day 21 confirmed the presence of vasogenic edema and tissue loss (see figs 2, 7 and 8). Infarct size as determined from $T_2$ maps was 89% smaller ($p<0.0005$) in interferon treated animals compared to controls.

To summarize, treatment with interferon-beta affords important neuroprotection to ischaemic brain. Already at 1 day after reperfusion, the amount of tissue at risk to undergo permanent infarction identified by the ADC data, is significantly smaller in interferon-treated animals than in control animals. Permanent damage as assessed by $T_2$ MRI shows the protection afforded at day 1 extends until at least 3 weeks after the insult, at which timepoint the volume of infarcted tissue is 89% smaller in treated animals compared to controls.

There was no significant difference between starting treatment 2 days prior to the insult, starting upon reperfusion or starting 4 hours after stroke onset, the latest timepoint tested, indicating a treatment window of 4 hours after onset of stroke and possibly longer.

Example 2

In this example, the number of experimental animals per group has been expanded compared to example 1. Example 2 further contains additional data. Statistical analysis in this example was performed on all animals tested in example 1 and example 2.

Materials and Methods

Animal model

Male Fischer rats (F344/Ico, Iffa-Credo Broekman, Someren, The Netherlands) aged 8-12 weeks were used in all experiments. Animals had free access to standard laboratory chow and water. Anesthesia was induced by i.p. injection of a mixture of 0.5 ml/kg fentanyl citrate (0.315 mg/ml) and fluanisone (10.0 mg/ml), and 0.5 ml/kg midazolam (5.0 mg/ml), followed by s.c. injection of 0.1 ml/kg (0.5 mg/ml) atropine sulfate and i.p. injection of 0.5 ml/kg gentamicinsulfate (10 mg/ml). The animals were endotracheally intubated and mechanically ventilated with O2/N2O (30/70 v/v). Body temperature was maintained at 37.0 ± 0.5 °C by means of a feedback-controlled heating pad. During surgery and NMR-measurements anesthesia was continued by adding 1-1.5% halothane to the $O_2/N_2O$ mixture. After surgery animals received 1 ml/kg buprenorfine s.c. (0.3 mg/ml).

Transient focal ischaemia was induced by unilateral tandem occlusion of the right common carotid artery (CCA) and middle cerebral artery (MCA) via a modification of the procedure as described by Brint et al. Briefly, a 2 mm hole was drilled just rostral to the oval foramen, exposing the right middle cerebral artery (MCA). After opening the dura and arachnoidea the MCA was transiently occluded using a vascular microclip (Codman). Perfusion was reinstated after 60 minutes under visual inspection by removing the clip.

Animals were treated with s.c. injections of 500.000 IU recombinant rat interferon- (~ 8 μg of protein) in 1 ml phosphate buffered saline (PBS) once daily until 7 days after reperfusion (n=17). Control animals (n=12) received s.c injections with saline, starting 2 days prior to surgery up to 7 days after reperfusion. Treated animals were divided into 4 groups, based on whether treatment started 2 days before surgery (n=3), upon reperfusion (n=3), 4 hours after stroke onset (n=4) or 6 hours after stroke onset (n=4). 3 control animals and 3 treated animals were killed at 24h after stroke onset an processed for histology. In 3 control animals and 5 treated animals (1 from group III and 4 from group IV) a tail vein was cannulated at day 1 for injection of the NMR-contrast agent dimegluminegadopentate (Gd-DTPA).

NMR experiments

A single-scan diffusion-trace MRI sequence (4 b values: 100-1780 s/mm2, repetition time (TR)=2s, echo time (TE)=100ms, number of transients (NT)=2) was used to generate quantified images of tissue water trace apparent diffusion coefficient (ADC). $T_2$-weighted images were acquired using a multi-echo sequence (8 TEs: 17.5ms + 7 x 17.5ms, TR=2s, NT=2). Before bolus injection a pre-contrast $T_1$-weighted dataset (TR=0.65 sec, TE=0.0125 sec, NT=2) was acquired and 15 min after bolus injection, identical but contrast-enhanced $T_1$-weighted images were acquired. Both the $T_2$-weighted and the diffusion-weighted datasets (3.2x3.2 cm2 FOV, 128x64 matrix, zero-filled to 256x256) consisted of 8 consecutive, 1.7-mm thick slices, with 0-mm slice gap. The pre- and post-contrast $T_1$-weighted images were acquired with a 128x128 matrix and zero-filled to 512x512. To minimize interference at the slice boundaries, slices were acquired in alternating order (1,3,5,7,2,4,6,8), thus maximizing the time between excitation of two neighbouring slices. For the diffusion-weighted imaging we used a double spin-echo pulse sequence with four pairs of bipolar gradients with specific predetermined signs in each of the three orthogonal directions as recently published. The combination of gradient

directions leads to cancellation of all off-diagonal tensor elements, effectively measuring the trace of the diffusion tensor. This provides unambiguous and rotationally invariant ADC values in one experiment, circumventing the need for three separate experiments. To minimize the inherently high sensitivity of diffusion-weighted imaging to motion, the acquisition was triggered to the respiration cycle. Diffusion-weighted and $T_2$-weighted-datasets were acquired at 1, 7 and 21 days after stroke onset.

Immunohistochemistry

The following mouse-α-rat mAb were used: OX-6 (αMHC class II; IgG1 isotype; Serotec, Oxford, UK), 1A29 (αICAM-1; IgG1 isotype; gift from Dr. T. Tamatani), 5F10 (αVCAM-1; IgG2a isotype; gift from Dr. R. Lobb, Biogen, Inc., Cambridge, USA) and HIS48 (αNeutrophil; IgGM isotype; Serotec, Oxford, UK). All stainings were performed at the dept. of Molecular Biology, VUMC, Amsterdam.

Rats were sacrificed at 24h hours after stroke onset. Brains were removed, snap-frozen and stored at -80°C. Cryostat sections (8 mm) were melted on gelatin coated glass slides and air-dried. Slides were fixed in acetone (10 min) and incubated with appropriate dilutions of mAb (2 μg/ml). As secondary antibody, a rabbit-a-mouse F(ab')2 conjugated to alkaline phosphatase (AF, Dako, Denmark) was used. Antibodies and conjugates were diluted in PBS with fetal calf serum (FCS). Omission of the primary antibody served as a negative control. After incubation with the primary antibody (60 min), slides were rinsed in PBS, incubated with conjugate (60 min) and washed again in PBS. AF activity was demonstrated by incubation with AS-BI substrate in 0.1 M Tris pH 8.7 buffer for 10 minutes. After mounting the sections in mouting medium they were examined with a NIKON Eclipse E800 microscope and recordings were made with a NIKON DXM1200 camera.

Data analysis

ADC and $T_2$ maps were generated by mono-exponential fitting using IDL (Research Systems, Boulder, USA). Parametric images were analysed in anatomic regions of interest using in-house software. Calculations of volumes of affected tissue were based on ipsilateral ADC or $T_2$ differing more than 20% (corresponding to >2x SD) from the mean value in the contralateral hemisphere.

Statistical analysis was done using a linear mixed effects model (in S-PLUS 2000 Professional Edition Release 3, Mathsoft Inc., USA), with Rat as random effect and Day and Treatment as fixed effects. After showing significance of all treatment effects, further statistical analysis was carried out using SPSS 10.0 (SPSS Inc, Chicago, USA). Reported p-values correspond to two-tailed Student's t-tests for the dichotomized comparisons (Treatment-start-2-days-prior vs Treatment-start-after-onset) or one-way ANOVA where appropriate, with correction for multiple comparisons.

Results

General Condition

After surgery, body weight of all animals showed an initial decrease in the first postoperative days, whereafter it increased.

In two treated animals a necrotic patch, about 7.5 mm in diameter, developed in the skin around the site of IFN-injection. The lesion was not painful to the touch and did not hinder the animals in feeding or other behavior. For the remaining days injection was continued at a different site, where, in these two animals, necrosis did not occur.

Magnetic Resonance Imaging

In control animals, both the cortex and a large part of the caudate putamen of the ipsilateral hemisphere were affected, whereas in IFN-treated animals cytotoxic cell swelling was restricted to a smaller area of the caudate putamen and to only a small rim of cortex, if at all, (Fig. 11). At this timepoint the volume of the lesion on ADC maps was ~70% smaller ($P<0.01$) in IFN-treated animals than in controls (Fig. 12). Also, the severity of tissue damage, as determined from the extent of ADC reduction, was 14% less ($P<0.001$) in IFN--treated animals than in control animals (Table 1). There was no significant difference in ADC data between starting IFN-treatment up to 6 hours after stroke had occured or starting treatment 2 days before surgery.

$T_2$ maps, calculated from $T_2$-weighted datasets, showed the presence of vasogenic oedema in the affected tissue, the volume of which was ~67% smaller ($P<0.05$) in IFN-treated animals compared to controls (Figs. 11 and 15). This difference was also demonstrated by the midline-shift caused by the swelling of brain tissue, which was absent or much less pronounced in IFN-treated animals than in controls.

We subsequently analysed the effect of starting IFN treatment 2 days before surgery or after stroke had occurred. In terms of the ADC data there was no

difference between prophylactic treatment and post-hoc treatment. In terms of the $T_2$ data, prophylactically treated animals had ~65% smaller lesion volumes on day 1 ($P<0.05$, Fig. 15) and the average lesion $T_2$-value was 12% lower ($P<0.05$, Table 1) than that of animals treated after stroke onset. From day 7 onwards, the effect of starting treatment up to 6 hours after stroke was similar to that of starting treatment 2 days before surgery.

On day 21, the last measurement day, ADC values had increased to supranormal values, which are indicative of vasogenic oedema and tissue loss (Figs. 11 and 12). The infarct size on ADC maps was ~80% smaller ($P<0.0001$) in IFN-treated animals than in controls. $T_2$ data acquired on day 21 confirmed the presence of vasogenic oedema and tissue loss (Fig. 11). Infarct size as determined from $T_2$ maps was ~85% smaller ($P<0.0001$) in IFN-treated animals than in controls (Fig. 15).

There was no significant difference between starting treatment 2 days prior to the insult, starting upon reperfusion or starting 4 or 6 hours after reperfusion, the latest timepoint tested, indicating a treatment window of 6 hours after onset of stroke and possibly longer.

The BBB is the functional sum of the endothelial cells, pericytes, astrocytes and extra-cellular matrix molecules that surround the blood flowing through the brain. Opening of the BBB may result from H/I related inflammation. IFN has been reported to prevent opening of the BBB, both in the rat EAE model and in MS patients. To study the effect of IFN on BBB integrity, the leakage of the intravascular contrast agent Gd-DTPA was determined at 24h after stroke onset by subtraction of pre- and post-contrast $T_1$-weighted images. The cortical BBB was better preserved in IFN- treated animals than in controls, as evidenced by a 49% decrease in Gd-DTPA-induced signal enhancement ($P<0.05$, Fig. 17).

Immunohistochemistry

The adhesion molecules ICAM-1 and VCAM-1 were expressed at low levels in the contralateral hemisphere of both control and IFN treated animals. At 24 hours after stroke onset there was a strong upregulation of both ICAM-1 and VCAM-1 in the ipsilateral hemisphere of control animals. This upregulation was reduced in IFN treated animals (Figs 18 and 19). A similar pattern was seen for MHC-II expression: No MHC-II was seen in the contralateral hemispheres of both control and IFN treated animals. In the ipsilateral hemisphere of control animals activated cells, expressing MHC-II, were present. This activation was prevented by IFN treatment (Fig 20). Immunostaining for HIS48 to detect the presence of neutrophilic infiltrate showed the absence of infiltration in the contralateral hemispheres of all animals. The ipsilateral hemisphere of control animals showed clear neutrophil inflammatory infiltrate. IFN treatment reduced infiltration (Fig 21).

Legends to the figures

**Fig 1. ADC maps of a slice through the center of the lesion acquired on day 1, 7 and 21.**
Top row: Animal treated with s.c. injections of saline.
Bottow row: Animal treated with s.c. injections of 500.000 IU rrIFN-$\beta$, starting at 3h after reperfusion.

**Fig 2. $T_2$ maps of a slice through the center of the lesion acquired on day 1, 7 and 21.**
Top row: Animal treated with s.c. injections of saline.
Bottow row: Animal treated with s.c. injections of 500.000 IU rrIFN-$\beta$, starting at 3h after reperfusion.

**Fig 3. Lesion volume of the control group and all treatment groups, on days 1, 7 and 21 as calculated from ADC maps.**

**Fig 4. Lesion volume of the control group versus all treatment groups together, on days 1, 7 and 21 as calculated from ADC maps.**

**Fig 5. Mean lesion ADC value of the control group and all treatment groups, on day 1 as calculated from ADC maps.** All values were decreased compared to normal tissue (0.00073 $mm^2/s$); control animals showed a stronger decrease in lesion ADC value.

**Fig 6. Mean lesion ADC value of the control group and all treatment groups, on days 7 and 21 as calculated from ADC maps.** All values were increased compared to normal tissue (0.00073 $mm^2/s$); control animals showed a stronger increase in lesion ADC value.

**Fig 7. Lesion volume of the control group and all treatment groups, on days 1, 7 and 21 as calculated from $T_2$ maps.**

**Fig 8. Lesion volume of the control group versus all treatment groups together, on days 1, 7 and 21 as calculated from $T_2$ maps.**

**Fig 9. Mean lesion $T_2$ value of the control group and all treatment groups, on days 1, 7 and 21 as calculated from $T_2$ maps.** All values were increased compared to normal tissue (0.055 s); control animals showed a stronger increase in lesion $T_2$ value.

**Fig 10. Mean lesion $T_2$ value of the control group versus all treatment groups together, on days 1, 7 and 21 as calculated from $T_2$ maps.** All values were increased compared to normal tissue (0.055 s); control animals showed a stronger increase in lesion $T_2$ value.

**Fig 11. ADC and $T_2$ maps of a slice through the center of the lesion acquired on day 1, 7 and 21.**

Top rows: ADC maps of an animal treated with s.c. injections of saline and of animal treated with s.c. injections of 500.000 IU rrIFN-beta, starting at 6h after stroke onset.

Bottom rows: $T_2$ maps of the same two animals.

**Fig 12. Lesion volume of the control group and all treatment groups, on days 1, 7 and 21 as calculated from ADC maps.** Since there was no significant difference between any of the IFN-treatment groups over time, the mean effect of IFN treatment is shown as IFN-mean. Asterisks denote significance of difference from control.

**Fig 13. Mean lesion ADC value of the control group and all treatment groups, on day 1 as calculated from ADC maps.** Irrespective of the size of the lesion, all mean lesion ADC values were decreased compared to normal tissue (0.00073 $mm^2/s$); control animals showed a stronger decrease in lesion ADC value.

**Fig 14. Mean lesion ADC value of the control group and all treatment groups, on days 7 and 21 as calculated from ADC maps.** Irrespective of the size of the lesion, all mean lesion ADC values were increased compared to normal tissue (0.00073 $mm^2/s$); control animals showed a stronger increase in lesion ADC value.

**Fig 15. Lesion volume of the control group and all treatment groups, on days 1, 7 and 21 as calculated from $T_2$ maps.** Since there was no significant difference between any of the IFN-treatment groups over time, the mean effect of IFN treatment is shown as IFN-mean. Asterisks denote significance of difference from control. Ampersand denotes significance of difference between prophylactic and post-hoc treatment.

**Fig 16. Mean lesion $T_2$ value of the control group and all treatment groups, on days 1, 7 and 21 as calculated from $T_2$ maps.** Irrespective of the size of the lesion, all mean lesion $T_2$ values were increased compared to normal tissue (0.054 s); control animals showed a stronger increase in lesion $T_2$ value.

**Fig 17. Effect of IFN on BBB integrity assessed 24h after stroke.** The integrity of the BBB was better preserved in the cortex of IFN_treated animals than in control animals as evidenced by a 49% decrease in signal GdDTPA-induced enhancement. Asterisk, $P<0.05$ versus ischaemic control; Bars represent means ± SD.

**Fig 18. Effect of IFN on ICAM-1 expression assessed 24h after stroke.** Shown are sections through the ipsi- and contralateral hemispheres of IFN-treated animals (top row) and control animals (bottom row). A low level of ICAM-1 expression was seen in the contralateral hemisphere of both control and IFN-treated animals. ICAM-1 expression was upregulated after the ischaemic episode in the ipsilateral hemisphere of control animals and the upregulation was reduced by IFN treatment.

**Fig 19. Effect of IFN on VCAM-1 expression assessed 24h after stroke.** Shown are sections through the ipsi- and contralateral hemispheres of IFN-treated animals (top row) and control animals (bottom row). A low level of

VCAM-1 expression was seen in the contralateral hemisphere of both control and IFN-treated animals. VCAM-1 expression was upregulated after the ischaemic episode in the ipsilateral hemisphere of control animals and the upregulation was reduced by IFN treatment.

**Fig 20. Effect of IFN on MHC-II expression assessed 24h after stroke.**
Shown are sections through the ipsi- and contralateral hemispheres of IFN-treated animals (top row) and control animals (bottom row). No MHC-II expression was seen in the contralateral hemisphere of both control and IFN-treated animals. MHC-II expression was upregulated after the ischaemic episode in the ipsilateral hemisphere of control animals and the upregulation was reduced by IFN treatment.

**Fig 21. Effect of IFN on neutrophil infiltration assessed 24h after stroke.**
Shown are sections through the ipsi- and contralateral hemispheres of IFN-treated animals (top row) and control animals (bottom row). No neutrophil infiltration was seen in the contralateral hemisphere of both control and IFN-treated animals. Neutrophils infiltrated the ipsilateral hemisphere of control animals and the infiltration was reduced by IFN treatment.

**Table 1. Mean lesion $T_2$ value of the control group and all treatment groups, on days 1, 7 and 21 as calculated from $T_2$ maps.** All values were creased compared to normal tissue (0.055 s); control animals showed a stronger increase in lesion $T_2$ value.
Mean lesion ADC and T2-values for all treatment groups on all days. The extent of ADC-decrease or $T_2$-increase is a measure for the severity of the lesion, irrespective of its size. The ischemia-induced decrease in ADC and increase in $T_2$ is attenuated by IFN treatment. * $P < 0.05$ vs Ischemic Control, ** $P < 0.01$ vs Ischemic Control, *** $P < 0.001$ vs Ischemic Control.

Table 1

| * = p < 0.05<br>** = p < 0.01<br>*** = p < 0.001 | | Day 1 | | Day 7 | | Day 21 | |
|---|---|---|---|---|---|---|---|
| | | ADC (x $10^{-5}mm^2/s$) | $T_2$ (msec) | ADC (x $10^{-5}mm^2/s$) | $T_2$ (msec) | ADC (x $10^{-5}mm^2/s$) | $T_2$ (msec) |
| | Healthy Control | 71.6 ± 4.0 | 53.4 ± 0.4 | 71.8 ± 2.5 | 54.5 ± 1.1 | 72.8 ± 1.4 | 53.6 ± 2.6 |
| | Ischemic Control | 48.8 ± 2.6 | 81.4 ± 6.1 | 127.1 ± 7.4 | 85.6 ± 6.1 | 133.8 ± 4.9 | 93.4 ± 11.3 |
| IFN-β | 2 Days prior | 56.3 ± 2.6 * | 68.5 ± 3.6 ** | 102.1 ± 1.2* | 73.3 ± 3.5 * | 112.5 ± 1.5* | 72.4 ± 1.7 * |
| IFN-β | Upon reperfusion | 56.8 ± 3.5 ** | 73.5 ± 7.5 * | 119.2 ± 7.6 | 70.1 ± 1.0 ** | 117.2 ± 5.8* | 80.3 ± 6.3 |
| IFN-β | 4 Hours after onset | 58.1 ± 3.8 ** | 81.0 ± 3.2 | 115.9 ± 3.2* | 78.7 ± 2.1 | 119.1 ± 1.3* | 80.2 ± 12.6 |
| IFN-β | 6 Hours after onset | 54.6 ± 4.3 * | 77.6 ± 2.6 | 115.8 ± 11.4* | 78.8 ± 9.3 | 119.9 ± 1.2* | 76.8 ± 10.6* |
| IFN-β | IFN-mean | 56.4 ± 3.5 *** | 75.8 ± 6.1 | 116.7 ± 7.1** | 76.6 ± 6.5 ** | 117.6 ± 7.9** | 77.9 ± 8.5** |

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## Claims

1. A method for reducing cellular damage in tissue that has suffered from or is suffering from hypoxia and/or ischaemia and/or inflammation in an individual comprising administering to said individual a dose of IFN type-I or a functional part, derivative and/or analogue thereof.
2. A method for the treatment of a hypoxia/ischaemia (H/I) related inflammation in an individual comprising administering to said individual a dose of IFN type-I or a functional part, derivative and/or analogue thereof.
3. A method according to claim 1 or claim 2, wherein said H/I related inflammation is the result of a shortage in blood supply.
4. A method according to any one of claims 1-3, wherein said H/I related inflammation is restricted to a part of the body of said individual.
5. A method according to claim 4, wherein said part, comprises the brain, the spinal cord, the heart, a transplanted organ and/or a limb.
6. A method according to claim 5, wherein said H/I related inflammation is restricted to a part of the brain and/or the heart.
7. A method according to claim 6, wherein said H/I related inflammation is restricted to a part of the brain.
8. A method according to any one of claims 1-7, wherein said H/I related inflammation is induced by an obstruction of a blood vessel.
9. A method for the treatment of a H/I related inflammation in an individual, said individual comprising at least one blood vessel obstruction causing ischaemia in tissue that is situated downstream from said obstruction, said method comprising administering to said individual a dose of IFN type-I or a functional part, derivative and/or analogue thereof.
10. A method for at least in part improving blood flow in post-ischaemic tissue comprising administering to an individual, having said tissue, IFN type-I or a functional part, derivative and/or analogue thereof.

11. A method for at least in part preventing cell death in post-ischaemic tissue comprising administering to an individual, having said tissue, IFN type-I or a functional part, derivative and/or analogue thereof.

12. Use of IFN type-I or a functional part, derivative and/or analogue thereof, for the preparation of a medicament for the treatment of H/I related inflammation.

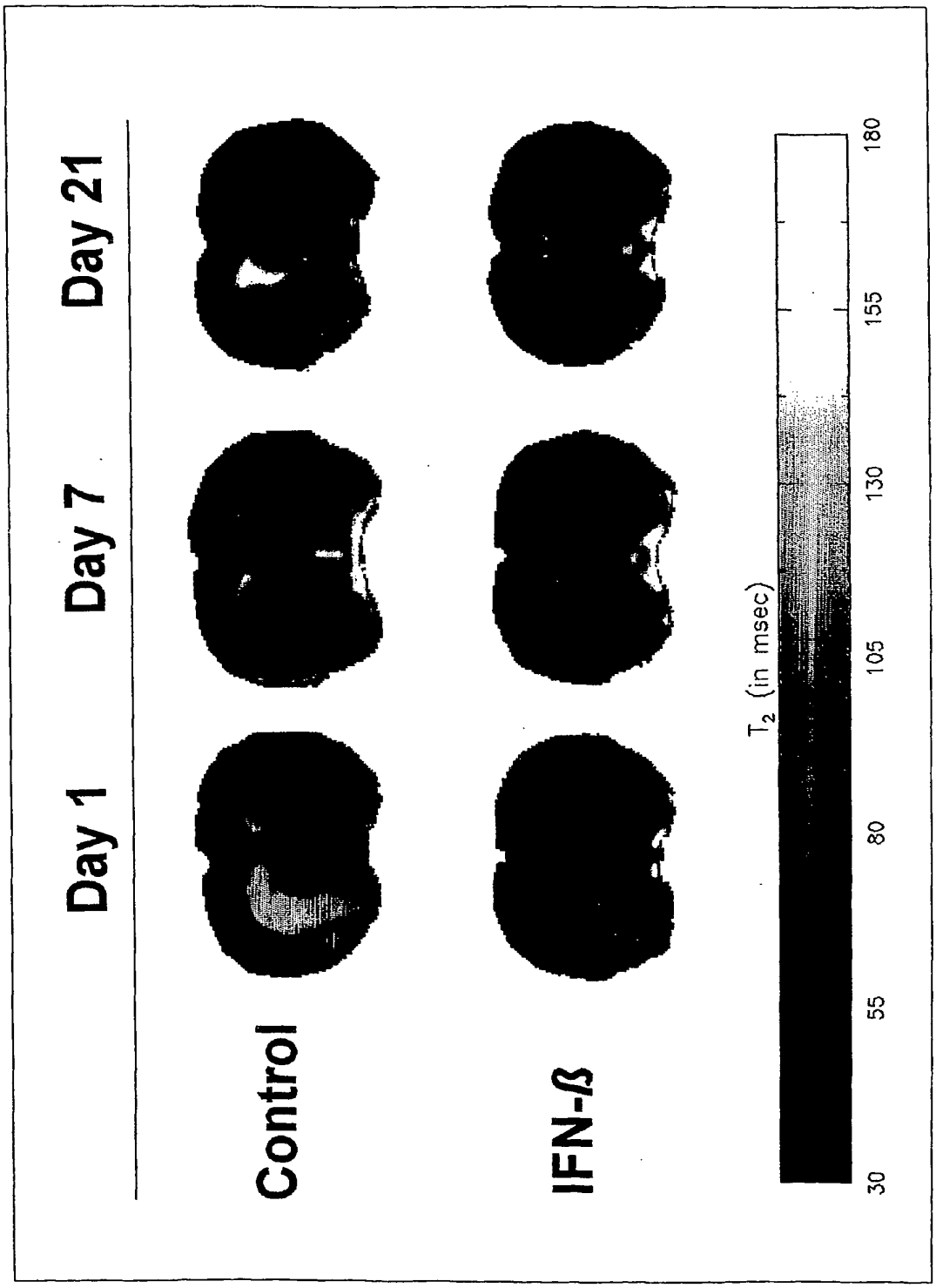
13. Use according to claim 11, wherein said inflammation is correlated with ischaemia.

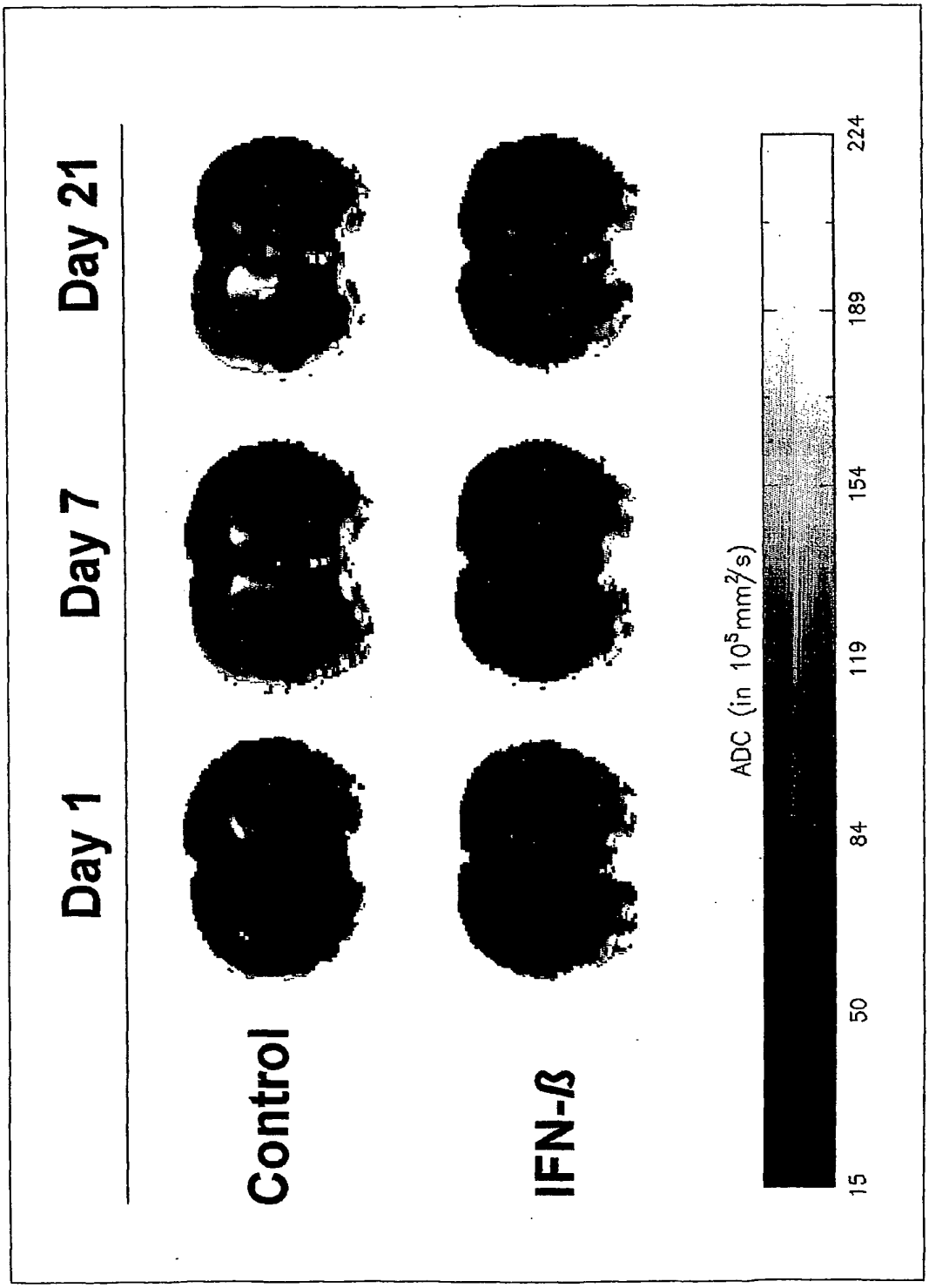
14. Use of IFN type-I or a functional part, derivative and/or analogue thereof for the preparation of a medicament for the treatment of impairment of blood flow recovery.

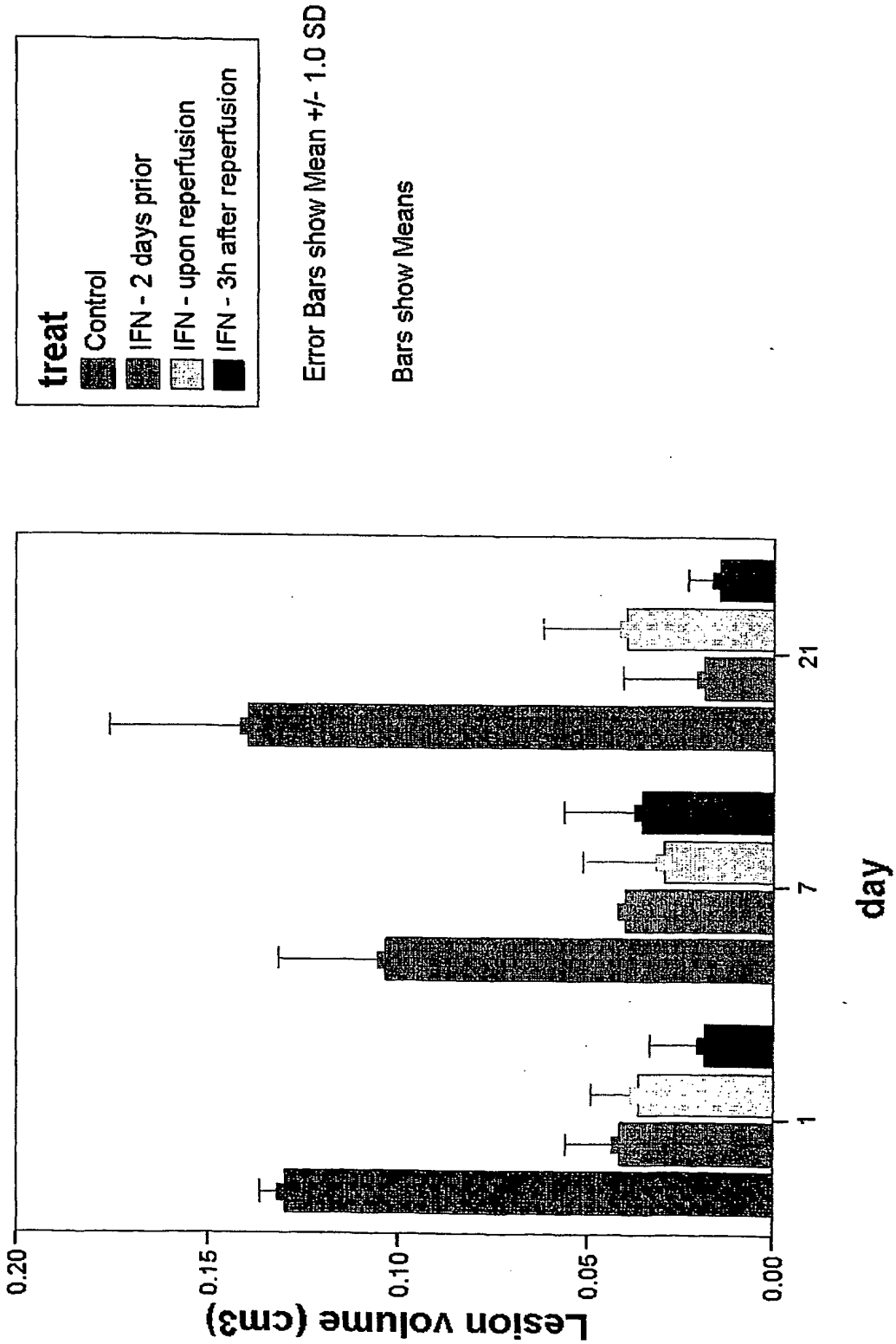
15. Use of IFN type-I or a functional part, derivative and/or analogue thereof for the preparation of a medicament for at least in part preventing cell death in post-ischaemic tissue.

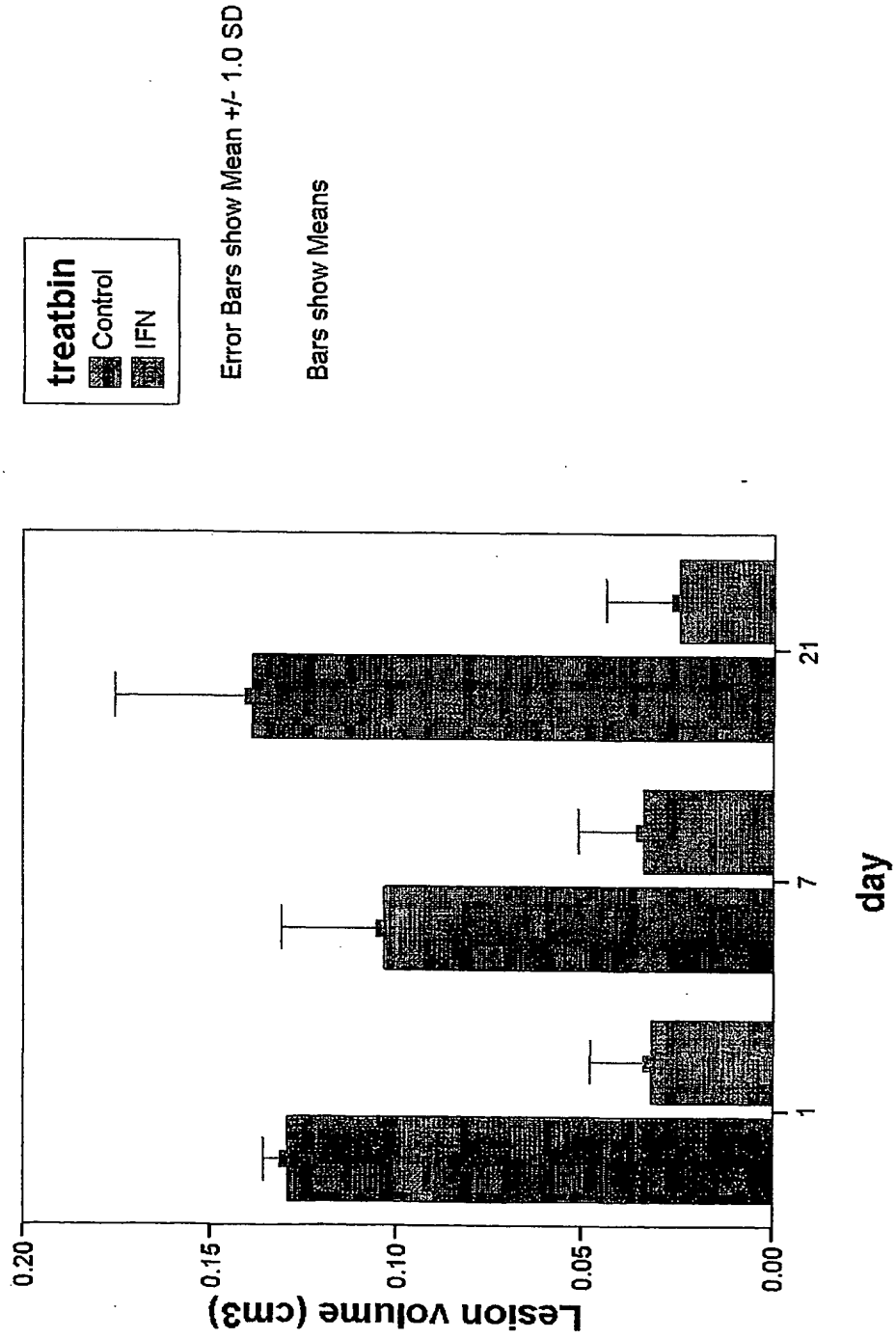
16. Use according to claim 14, wherein said impairment is in a capillary vessel

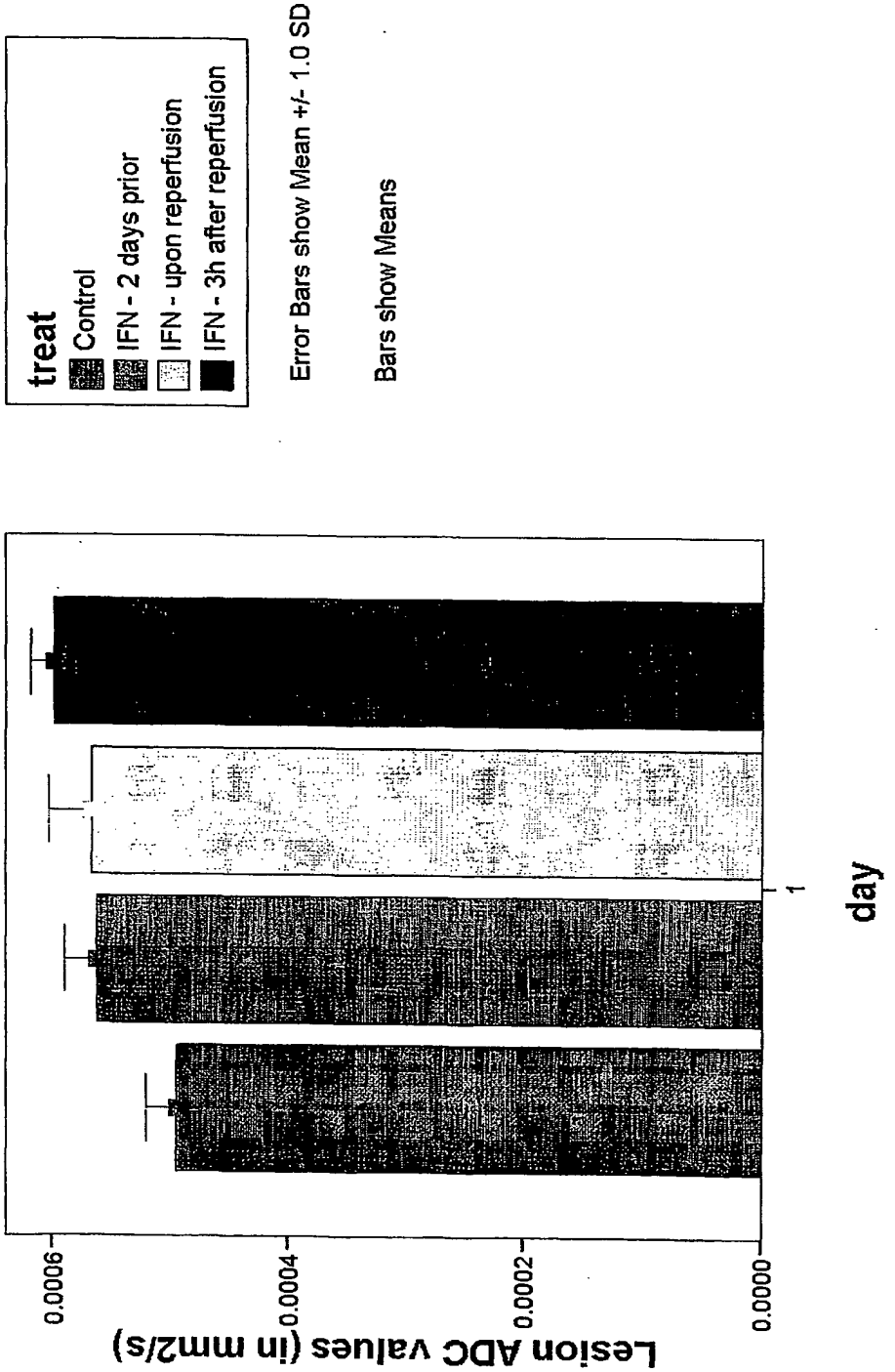
17. Use according to claim 14 or claim 16, wherein said impairment is in the brain.

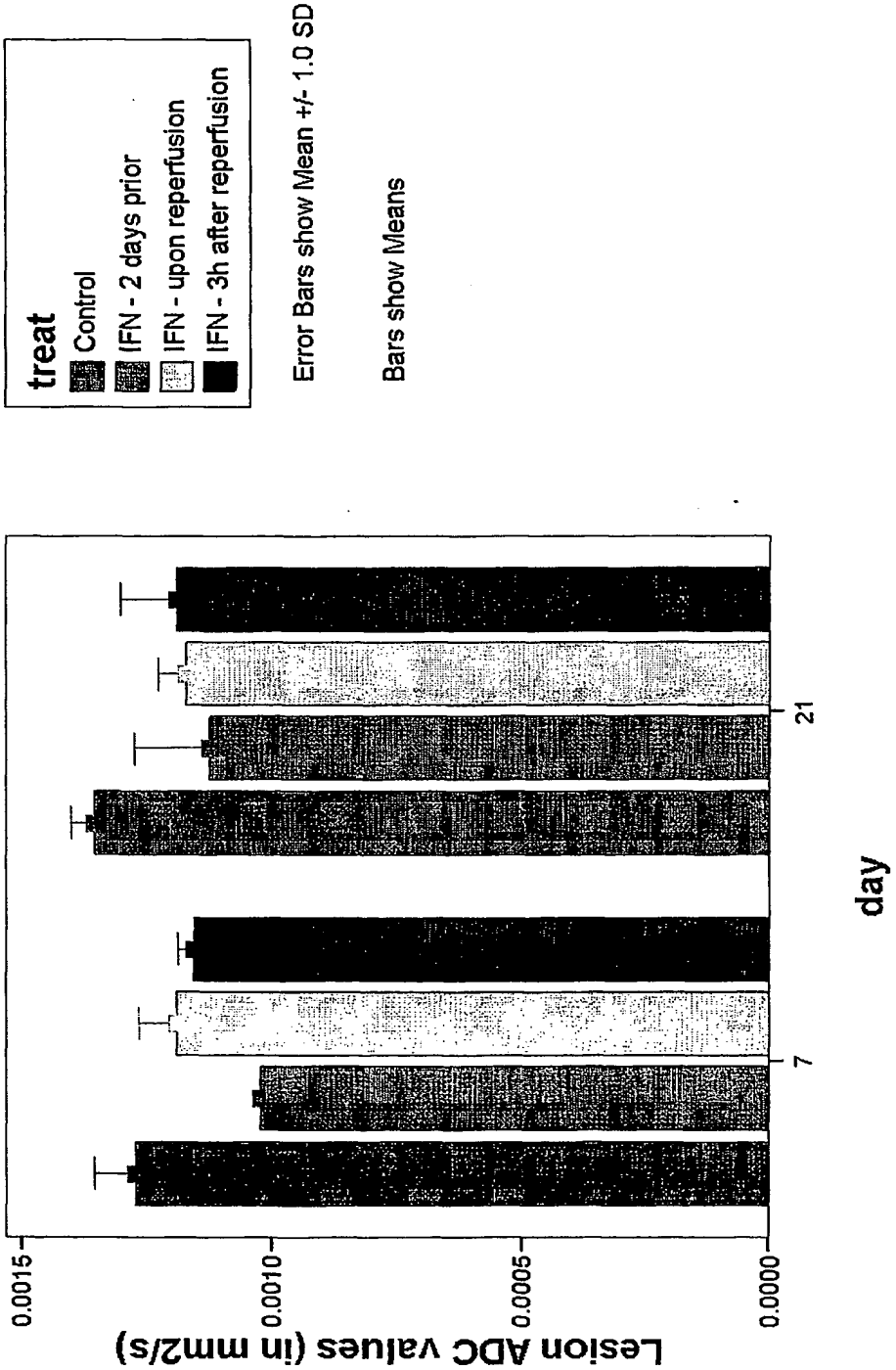

Day 1
Day 7
Day 21
Control
IFN-β
$T_2$ (in msec)
30
55
80
105
130
155
180

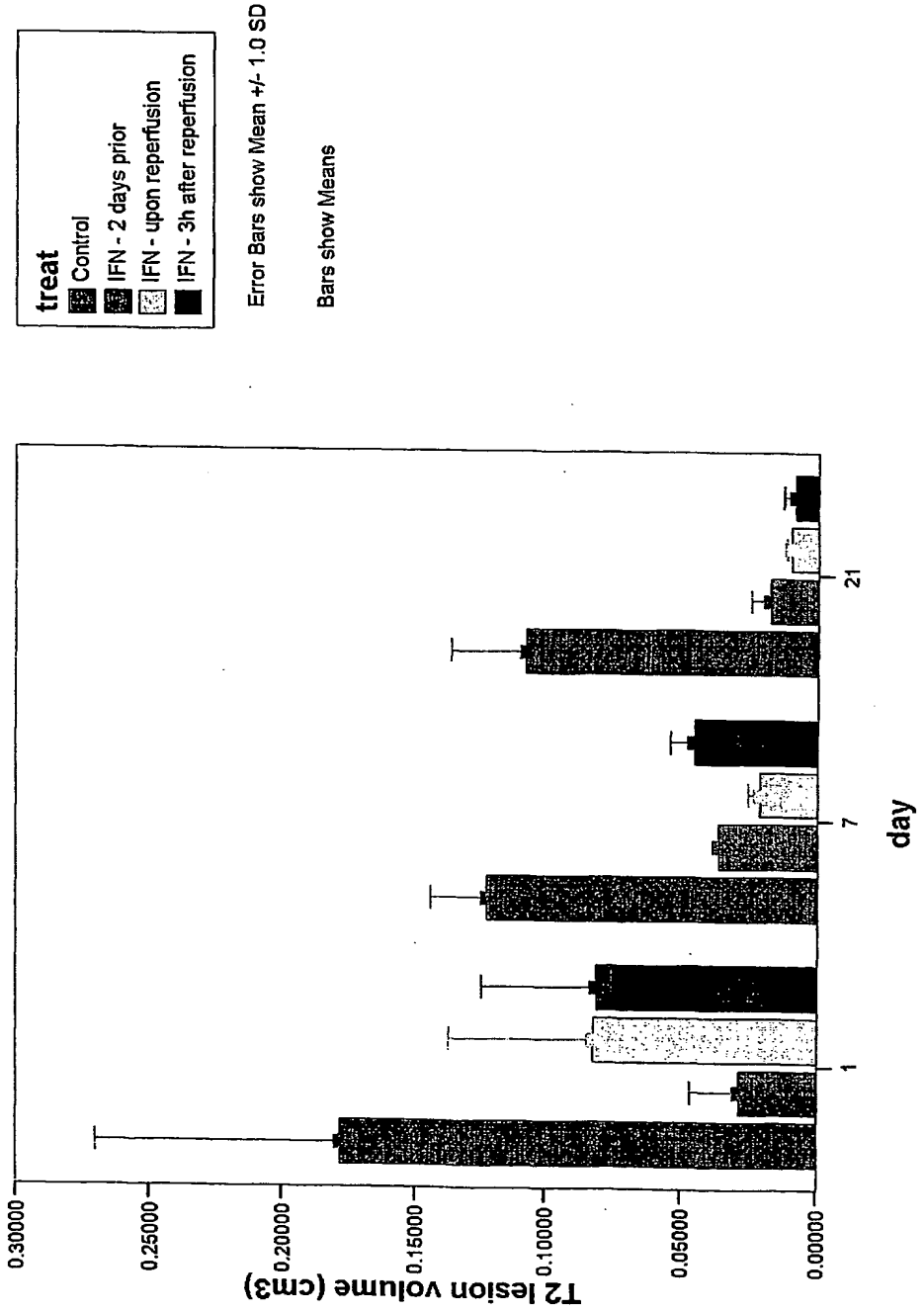

Day 1
Day 7
Day 21
Control
IFN-β
ADC (in $10^5 mm^2/s$)
15
50
84
119
154
189
224

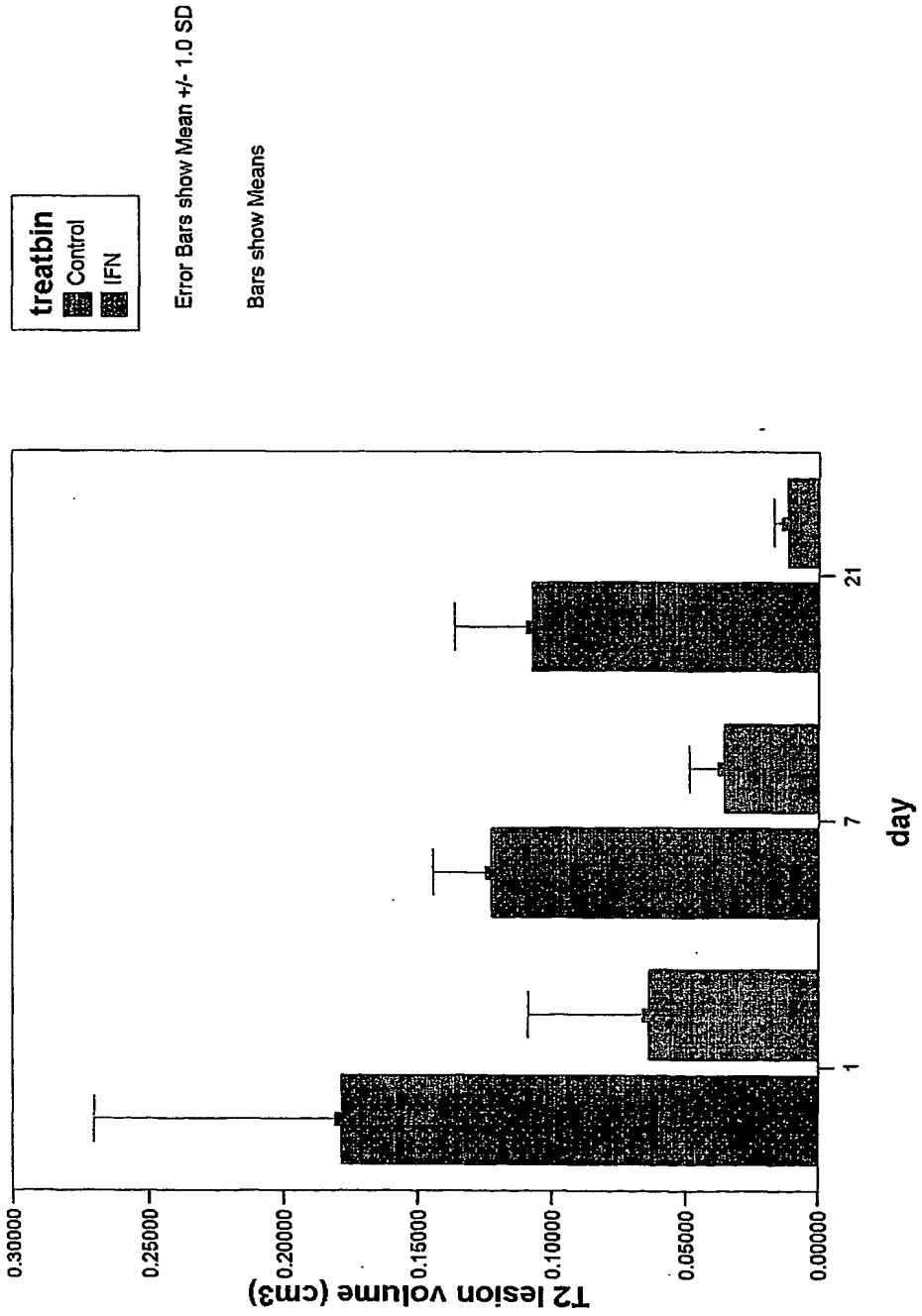

treat
Control
IFN - 2 days prior
IFN - upon reperfusion
IFN - 3h after reperfusion
Error Bars show Mean +/- 1.0 SD
Bars show Means
Lesion volume (cm3)
0.20
0.15
0.10
0.05
0.00
1
7
21
day

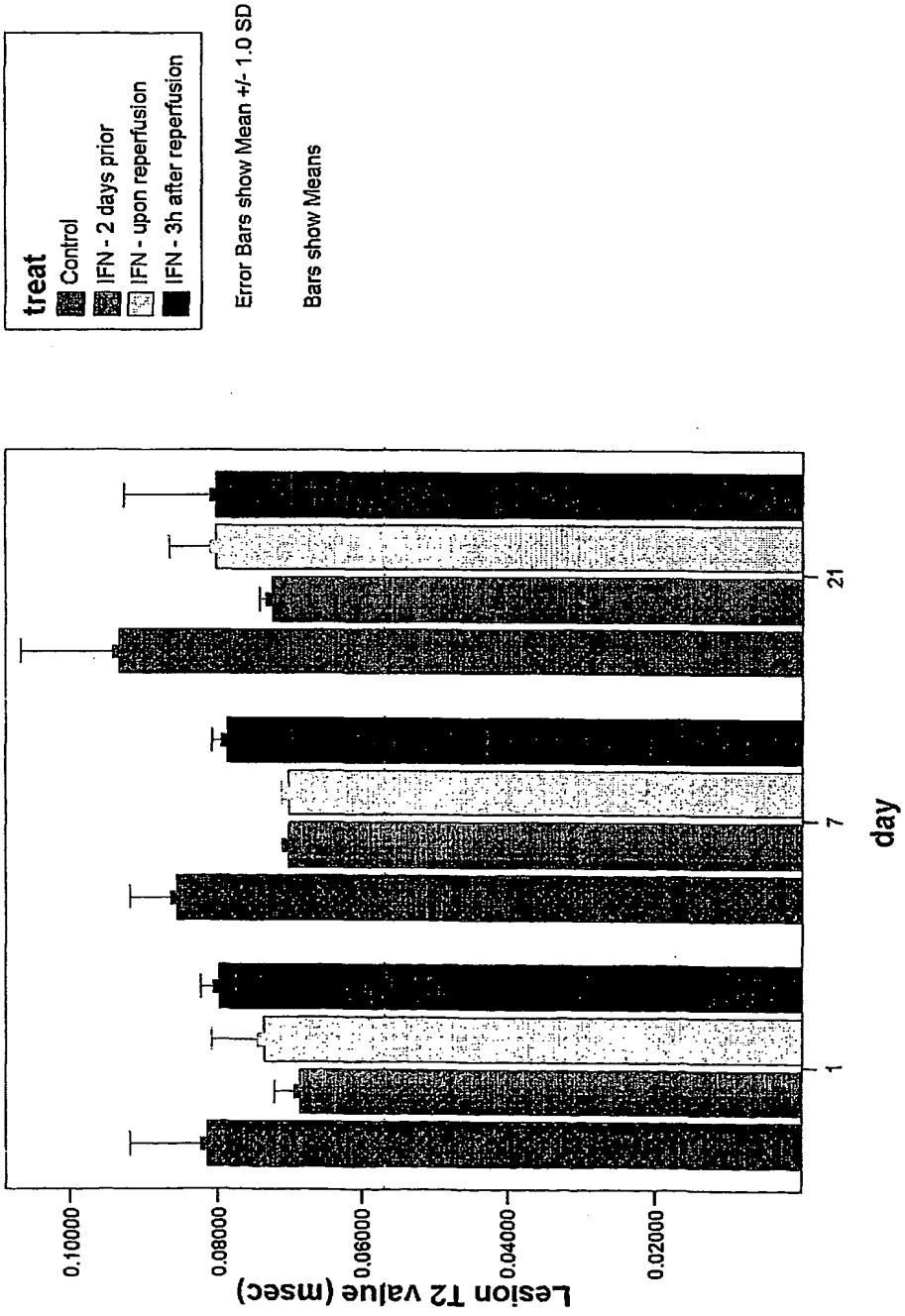

treatbin
Control
IFN
Error Bars show Mean +/- 1.0 SD
Bars show Means
Lesion volume (cm3)
0.20
0.15
0.10
0.05
0.00
1
7
21
day

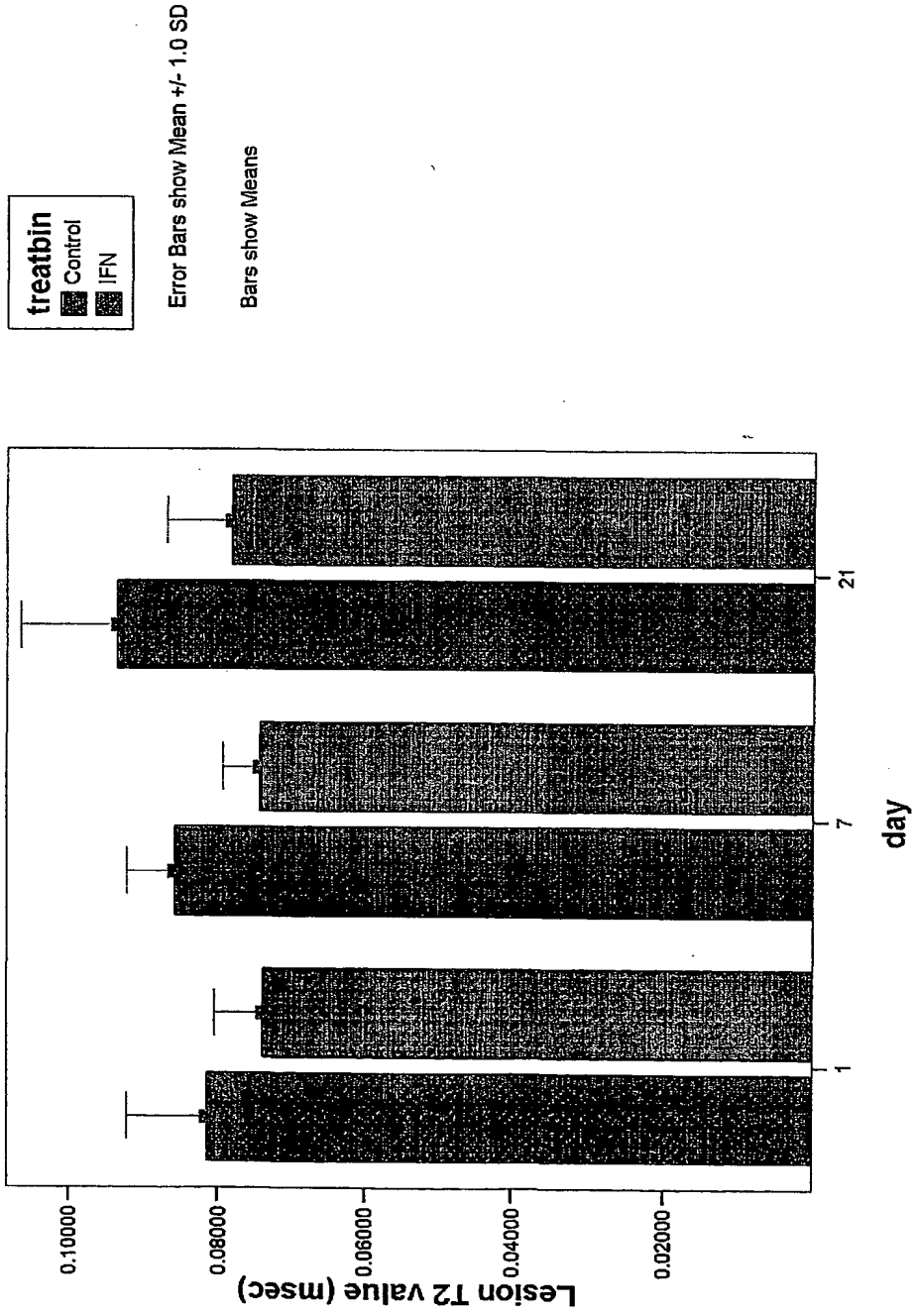

treat
Control
IFN - 2 days prior
IFN - upon reperfusion
IFN - 3h after reperfusion
Error Bars show Mean +/- 1.0 SD
Bars show Means
Lesion ADC values (in mm2/s)
0.0006
0.0004
0.0002
0.0000
1
day

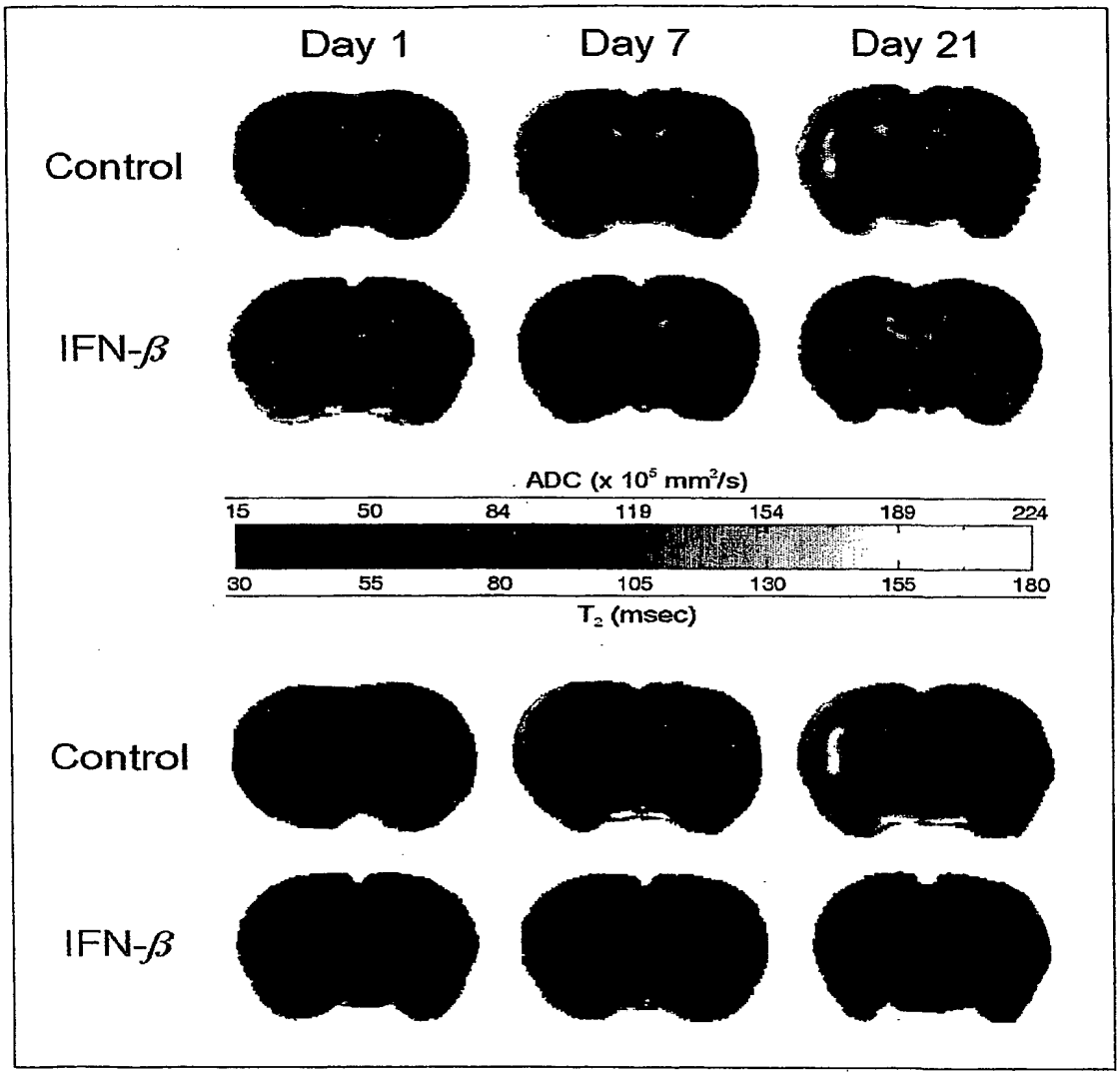

treat
Control
IFN - 2 days prior
IFN - upon reperfusion
IFN - 3h after reperfusion
Error Bars show Mean +/- 1.0 SD
Bars show Means
Lesion ADC values (in mm2/s)
0.0015
0.0010
0.0005
0.0000
7
21
day

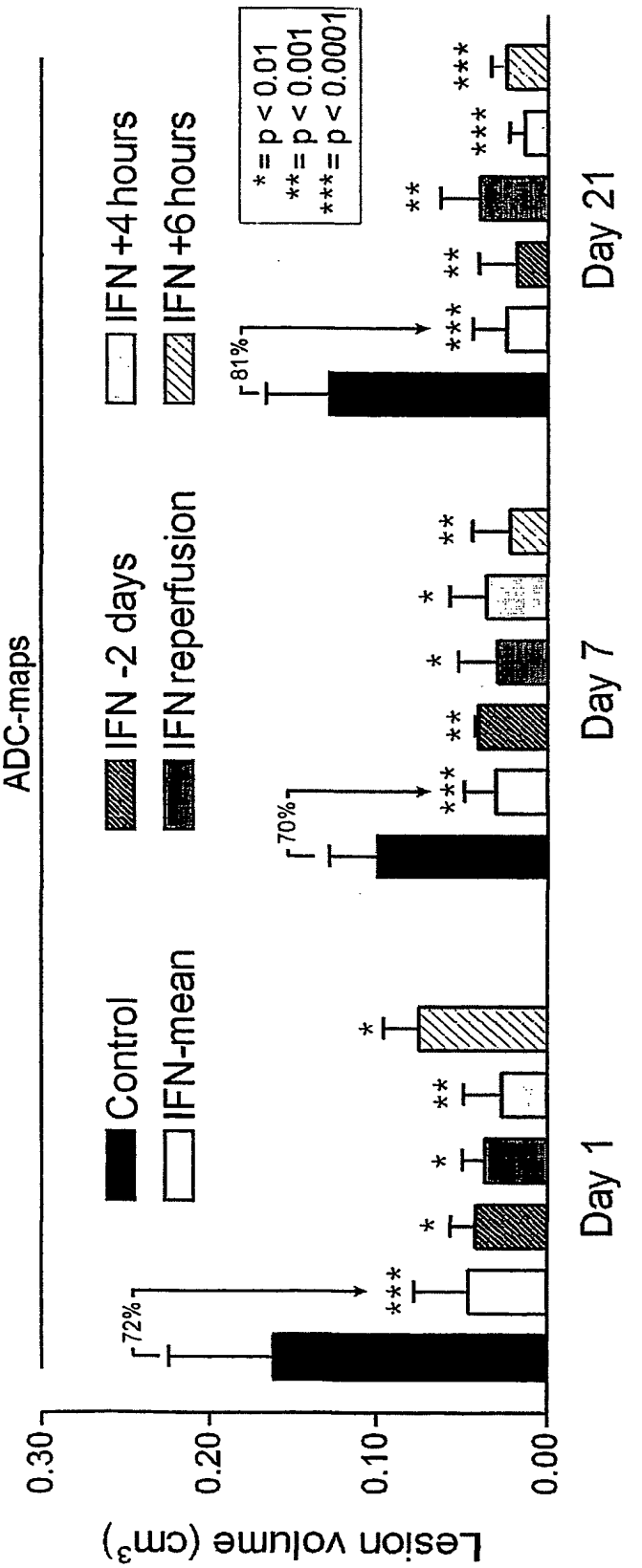

treat
Control
IFN - 2 days prior
IFN - upon reperfusion
IFN - 3h after reperfusion
Error Bars show Mean +/- 1.0 SD
Bars show Means
T2 lesion volume (cm3)
0.30000
0.25000
0.20000
0.15000
0.10000
0.05000
0.00000
1
7
21
day

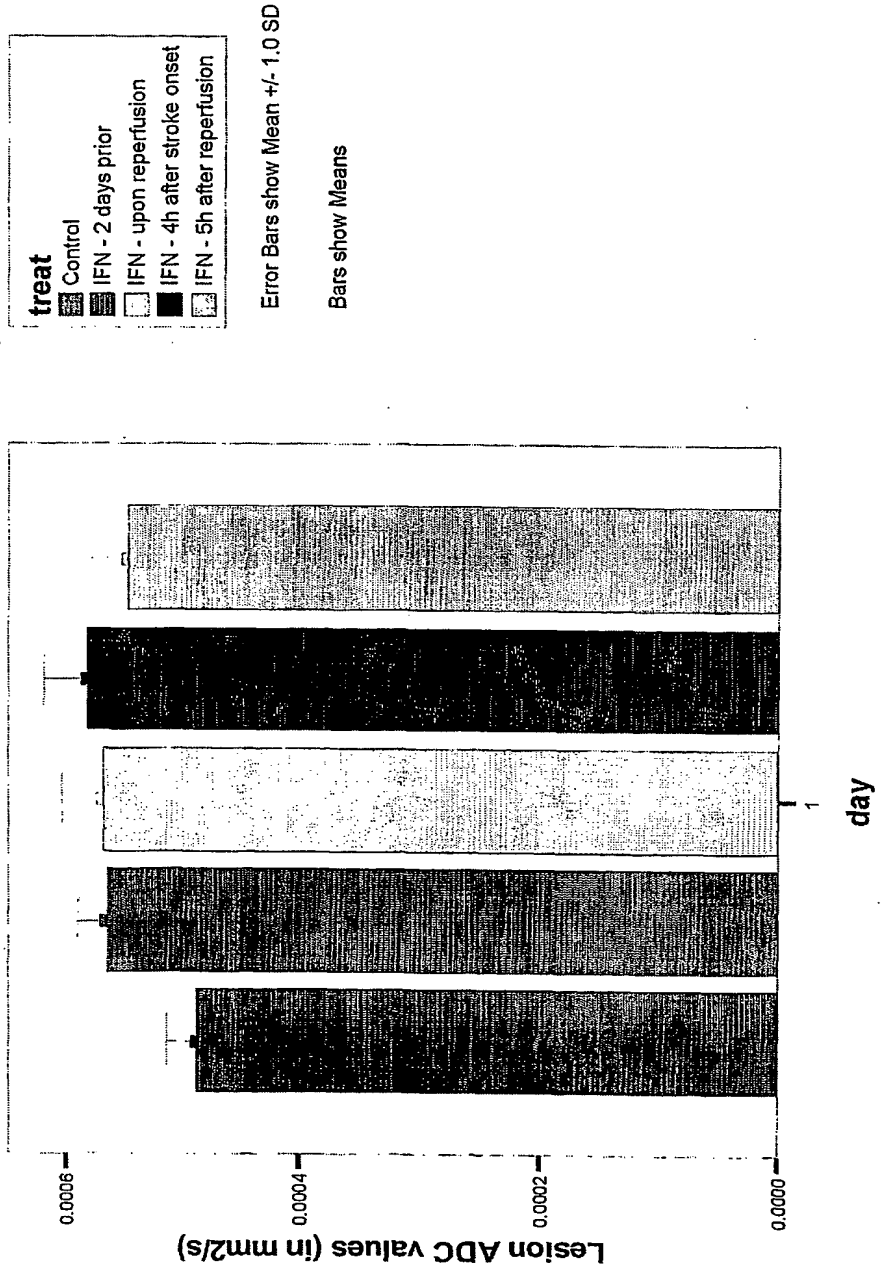

treatbin
Control
IFN
Error Bars show Mean +/- 1.0 SD
Bars show Means
T2 lesion volume (cm3)
0.30000
0.25000
0.20000
0.15000
0.10000
0.05000
0.00000
1
7
21
day

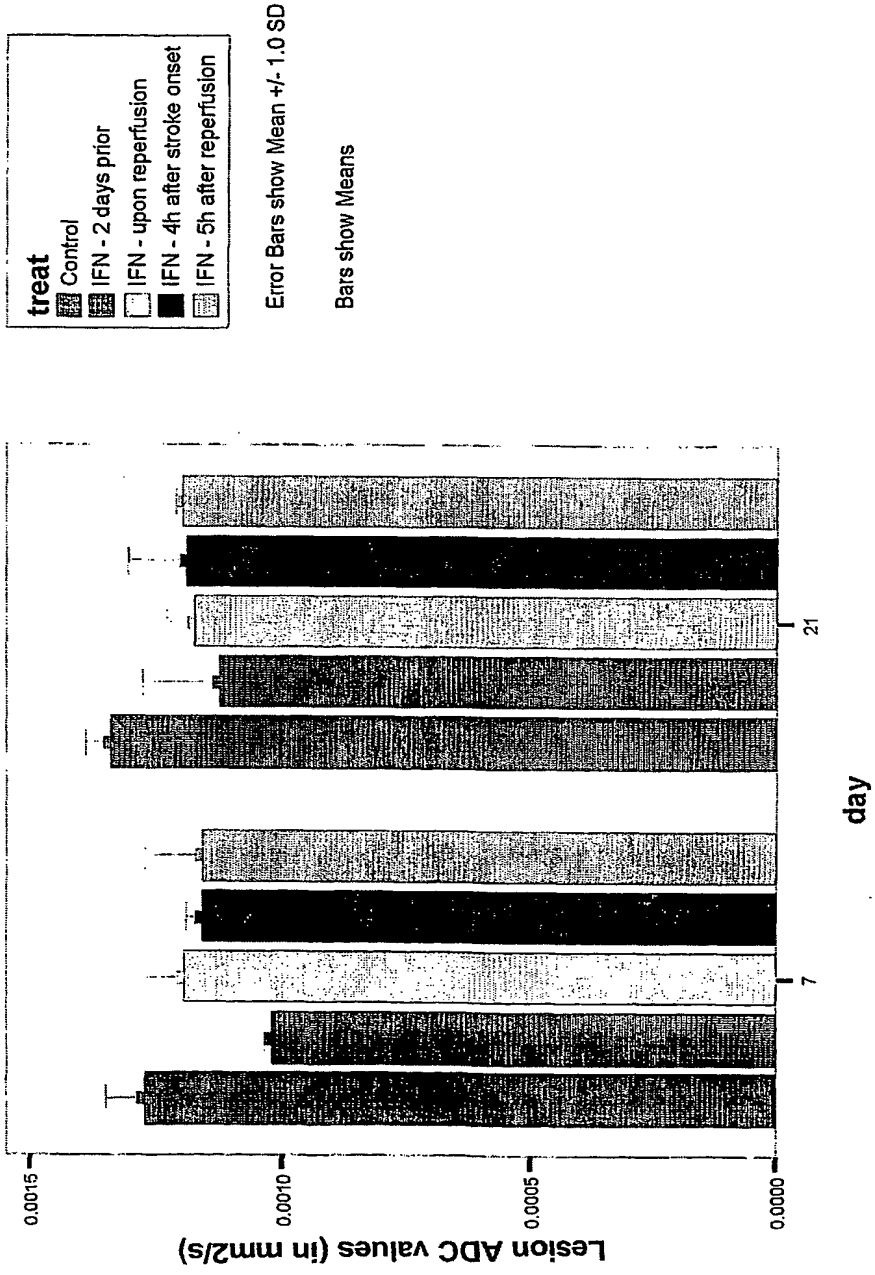

treat
Control
IFN - 2 days prior
IFN - upon reperfusion
IFN - 3h after reperfusion
Error Bars show Mean +/- 1.0 SD
Bars show Means
Lesion T2 value (msec)
0.10000
0.08000
0.06000
0.04000
0.02000
1
7
21
day

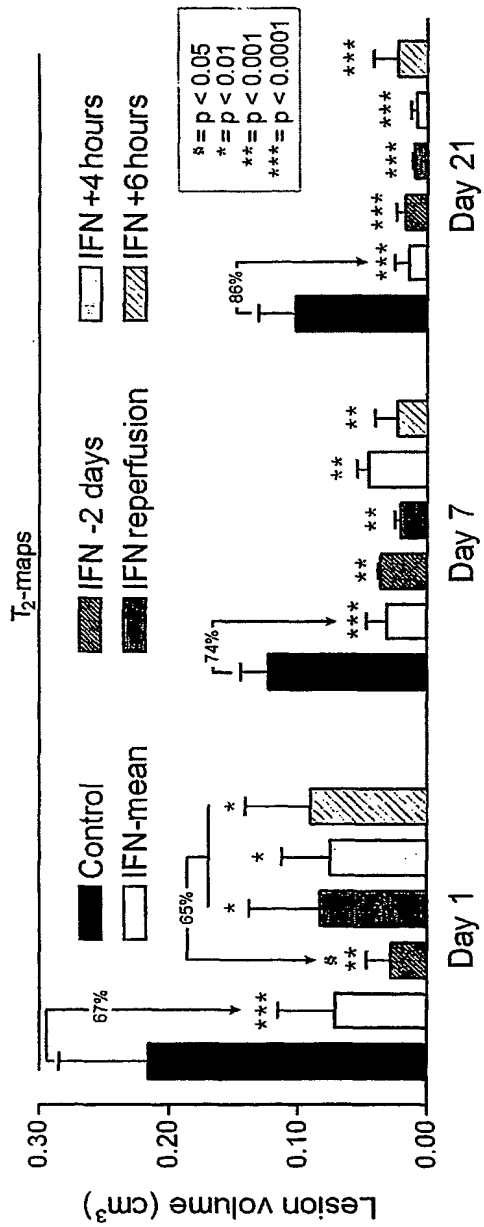

treatbin
Control
IFN
Error Bars show Mean +/- 1.0 SD
Bars show Means
Lesion T2 value (msec)
0.10000
0.08000
0.06000
0.04000
0.02000
1
7
21
day

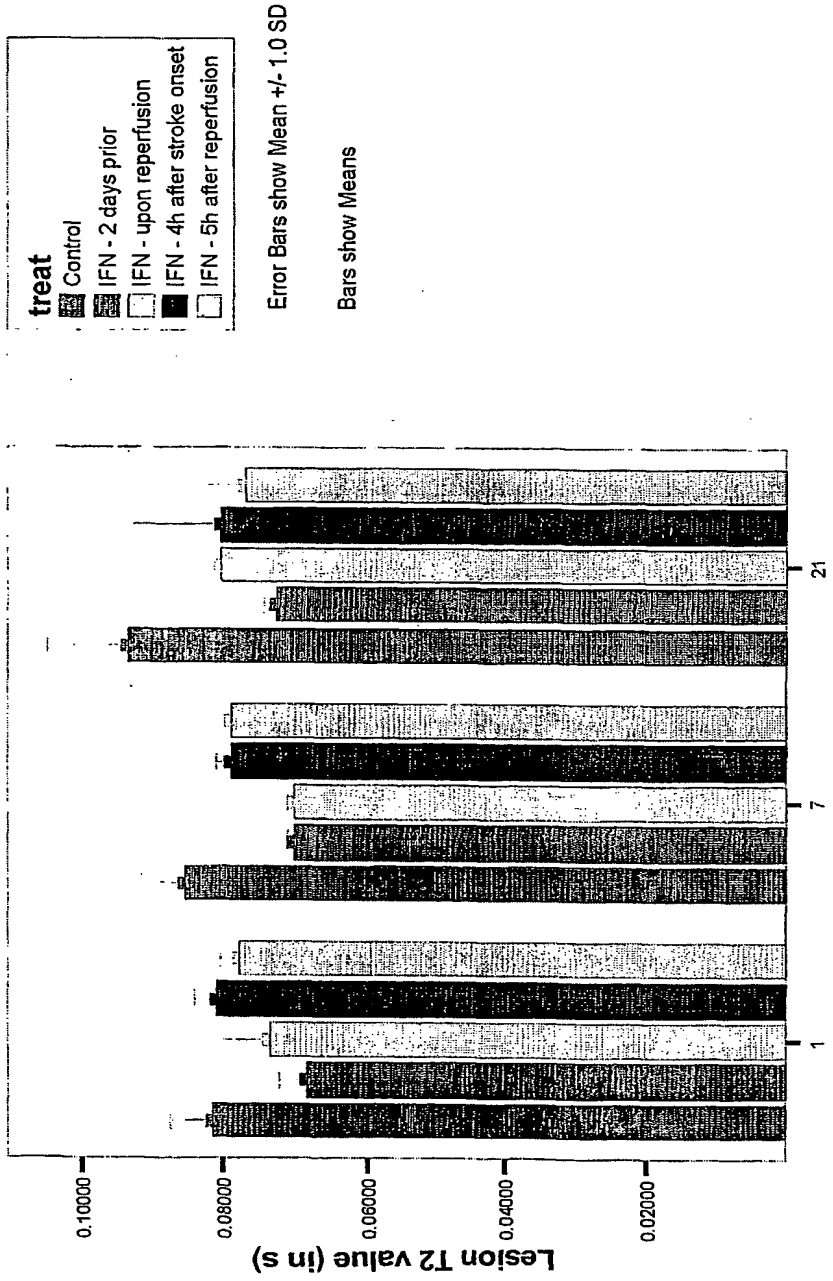

Day 1
Day 7
Day 21
Control
IFN-β
ADC (x 10⁵ mm²/s)
15 50 84 119 154 189 224
30 55 80 105 130 155 180
T₂ (msec)
Control
IFN-β

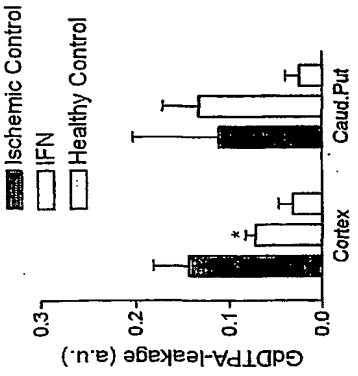

ADC-maps
Control
IFN-mean
IFN -2 days
IFN reperfusion
IFN +4 hours
IFN +6 hours
* = p < 0.01
** = p < 0.001
*** = p < 0.0001
Lesion volume (cm³)
0.30
0.20
0.10
0.00
72%
70%
81%
Day 1
Day 7
Day 21

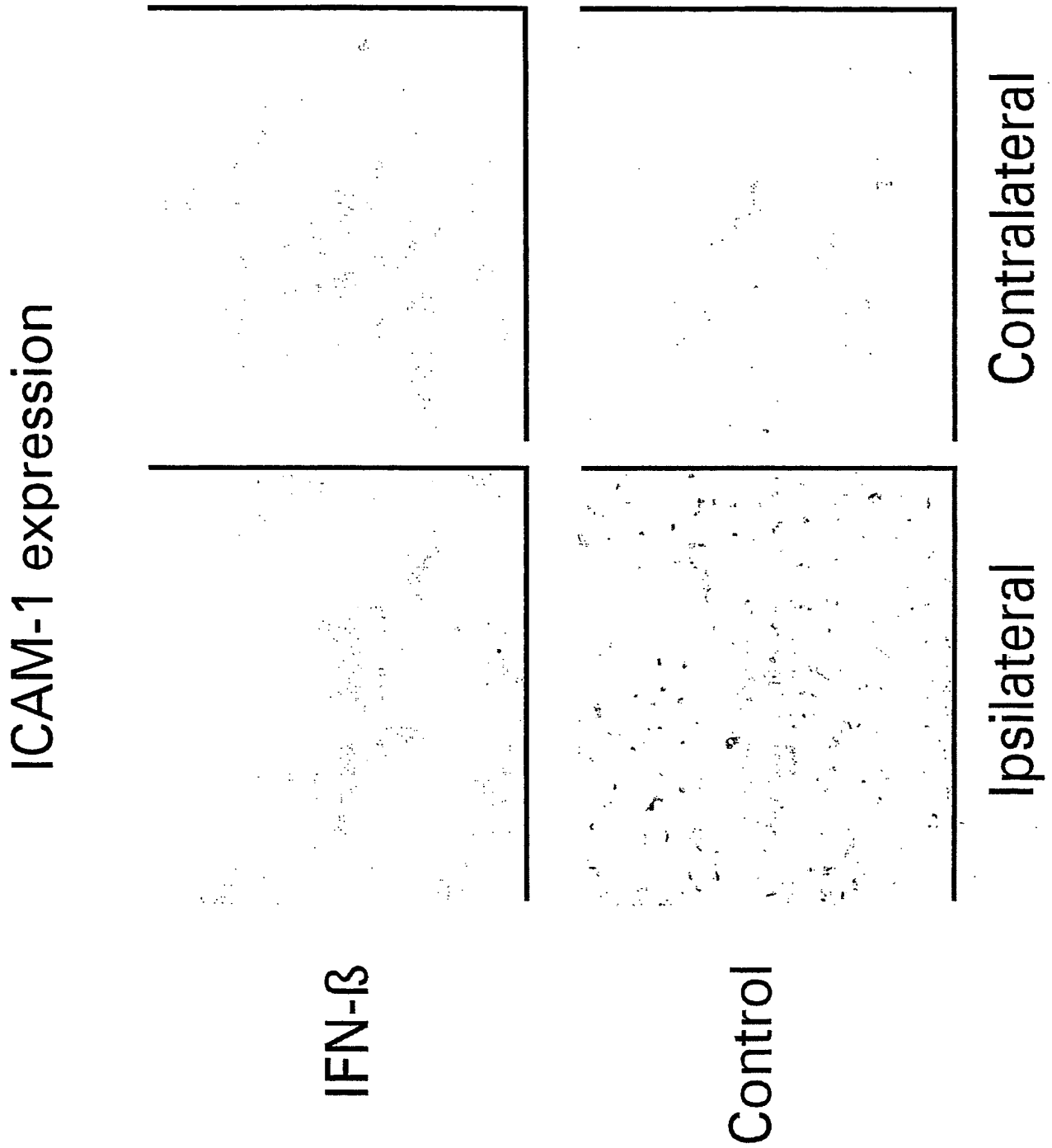

treat
Control
IFN - 2 days prior
IFN - upon reperfusion
IFN - 4h after stroke onset
IFN - 5h after reperfusion
Error Bars show Mean +/- 1.0 SD
Bars show Means
Lesion ADC values (in mm2/s)
0.0006
0.0004
0.0002
0.0000
1
day

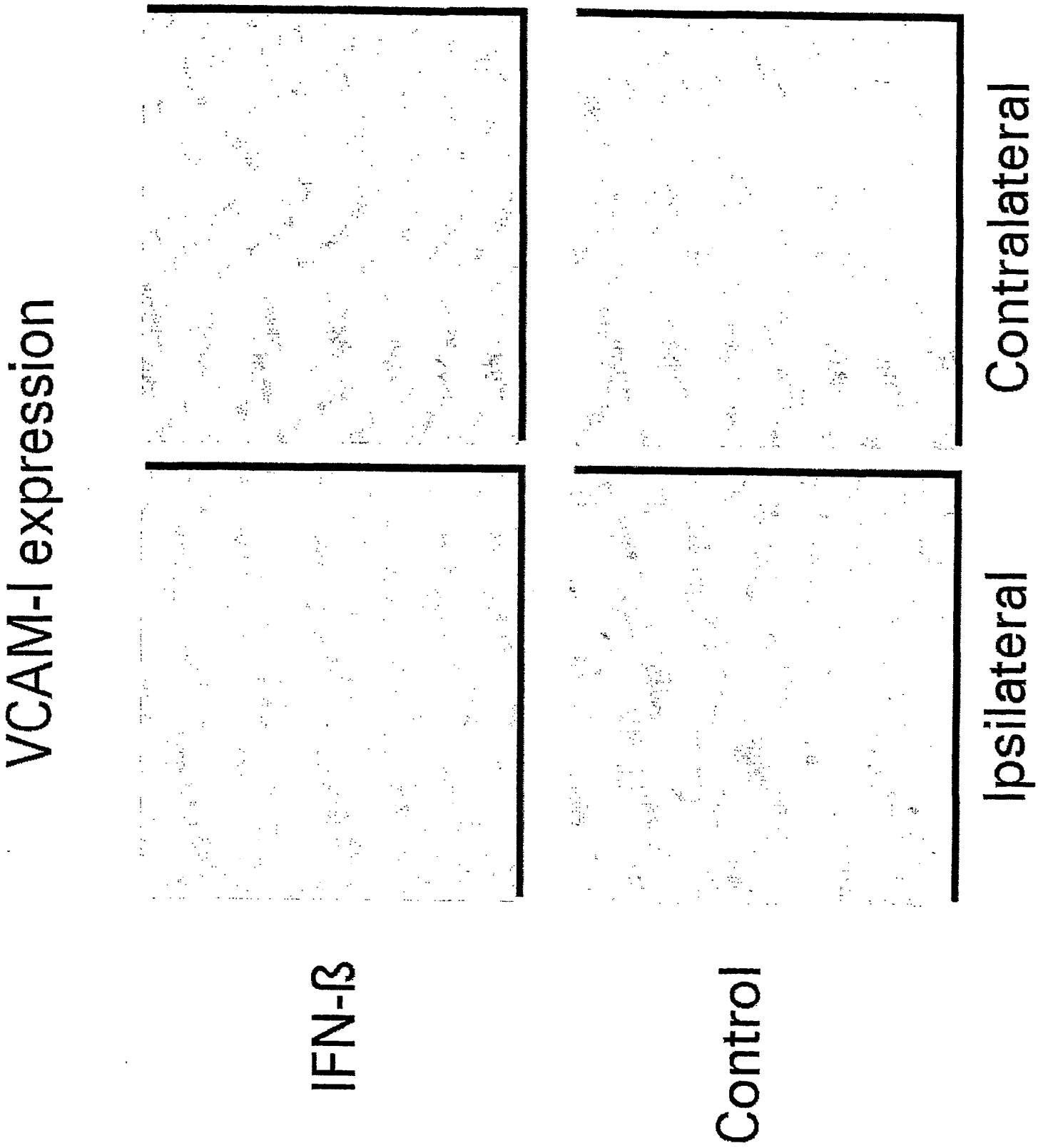

treat
Control
IFN - 2 days prior
IFN - upon reperfusion
IFN - 4h after stroke onset
IFN - 5h after reperfusion
Error Bars show Mean +/- 1.0 SD
Bars show Means
Lesion ADC values (in mm2/s)
0.0015
0.0010
0.0005
0.0000
7
21
day

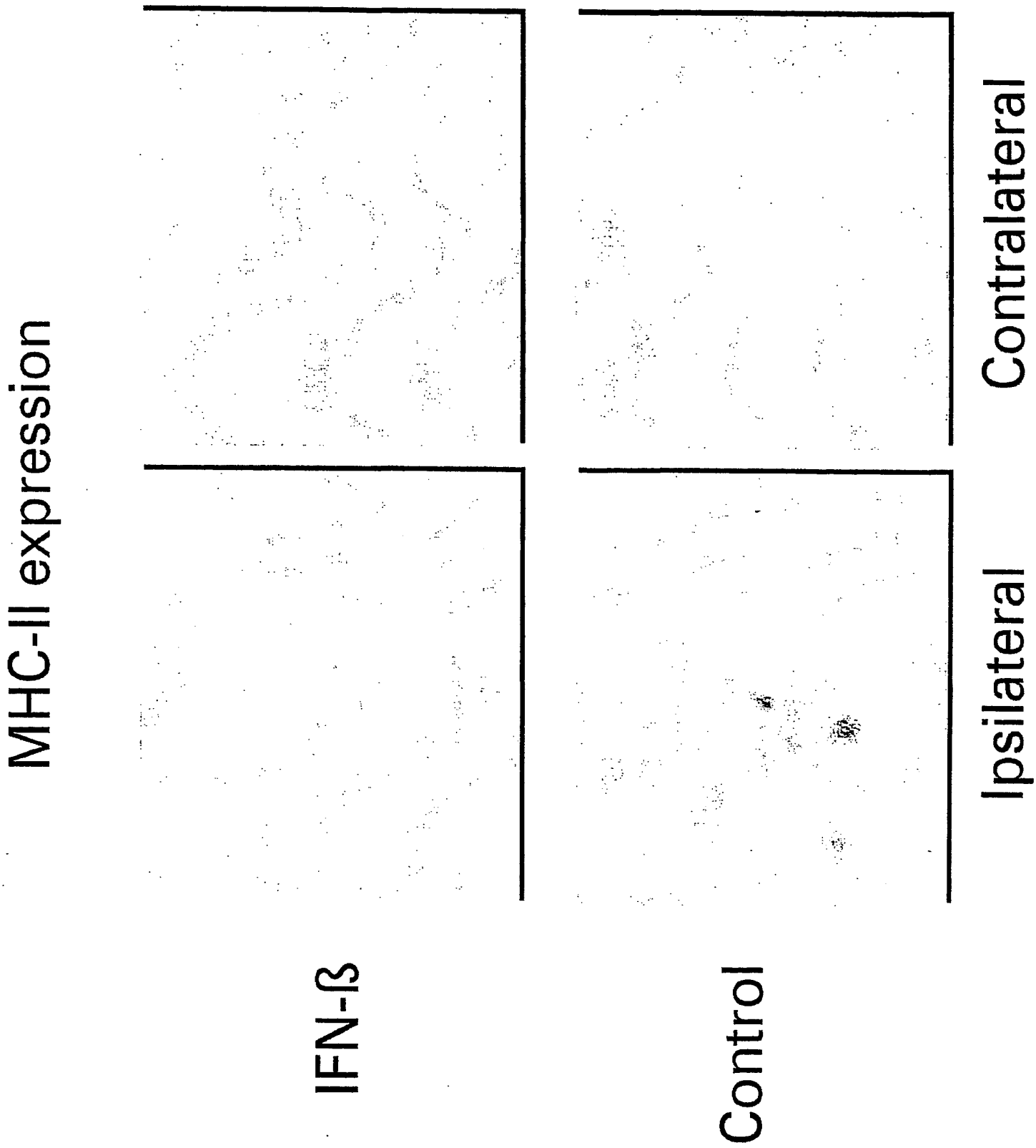

Lesion volume (cm³)
0.30
0.20
0.10
0.00
T2-maps
Control
IFN-mean
IFN -2 days
IFN reperfusion
IFN +4 hours
IFN +6 hours
Day 1
Day 7
Day 21
67%
65%
74%
86%
$ = p < 0.05
* = p < 0.01
** = p < 0.001
*** = p < 0.0001

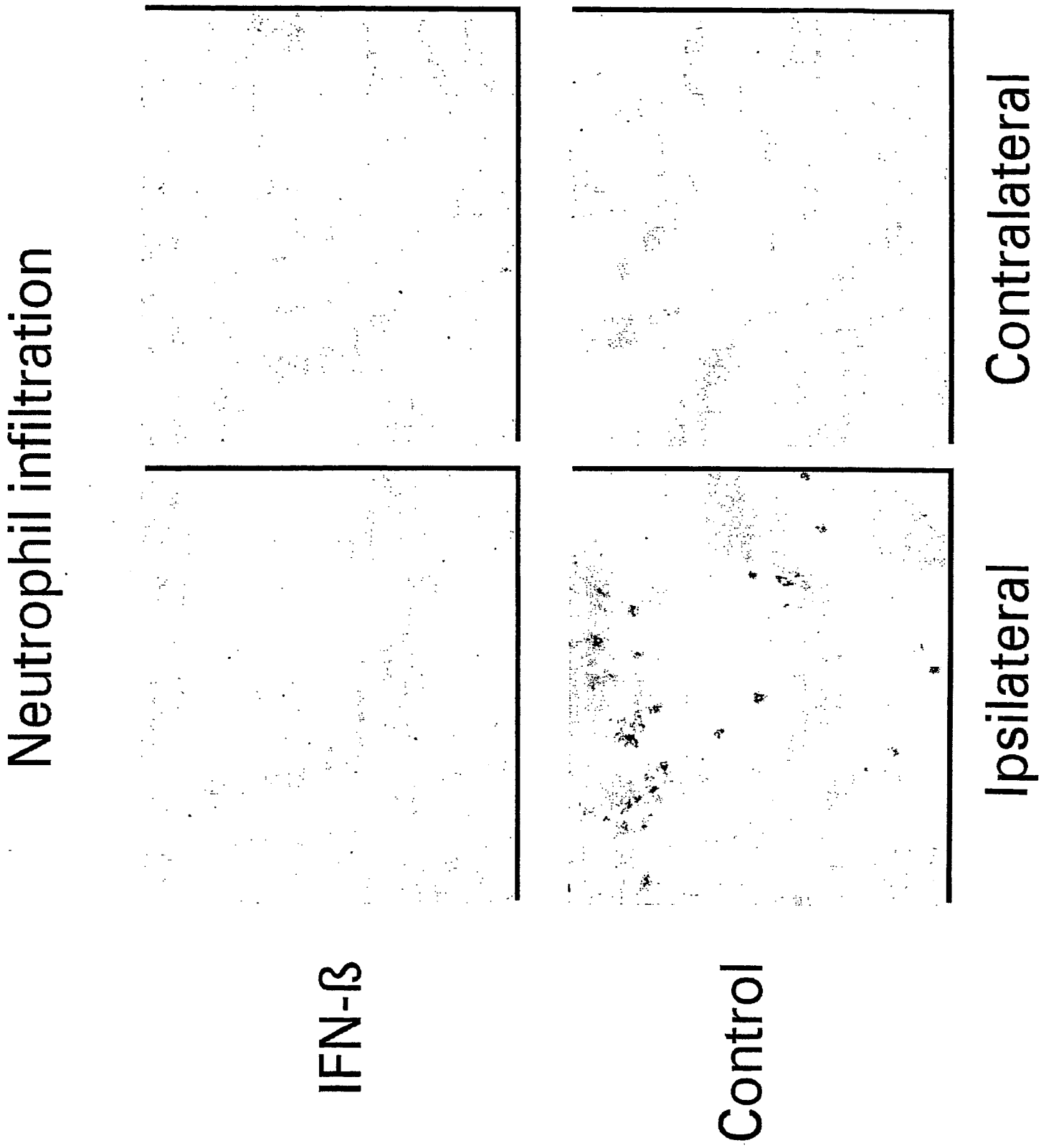

treat
Control
IFN - 2 days prior
IFN - upon reperfusion
IFN - 4h after stroke onset
IFN - 5h after reperfusion
Error Bars show Mean +/- 1.0 SD
Bars show Means
Lesion T2 value (in s)
0.10000
0.08000
0.06000
0.04000
0.02000
1
7
21


Ischemic Control
IFN
Healthy Control
GdDTPA-leakage (a.u.)
0.3
0.2
0.1
0.0
*
Cortex
Caud.Put


ICAM-1 expression
IFN-ß
Control
Ipsilateral
Contralateral


VCAM-I expression
IFN-β
Control
Contralateral
Ipsilateral


MHC-II expression
IFN-ß
Control
Ipsilateral
Contralateral


Neutrophil infiltration
IFN-ß
Control
Contralateral
Ipsilateral